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(71) Applicant *(for all designated States except US)*: LEUKOSITE, INC. [US/US]; 215 First Street, Cambridge, MA 02142 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: SCHWENDER, Charles, F. [US/US]; 143 Pine Street, Dover, MA 02030 (US). SHROFF, Hitesh, N. [US/US]; One Fairview Avenue, Bedford, MA 01730 (US).

(74) Agents: BROOK, David, E. et al.; Hamilton, Brook, Smith & Reynolds, P.C., Two Militia Drive, Lexington, MA 02173 (US).

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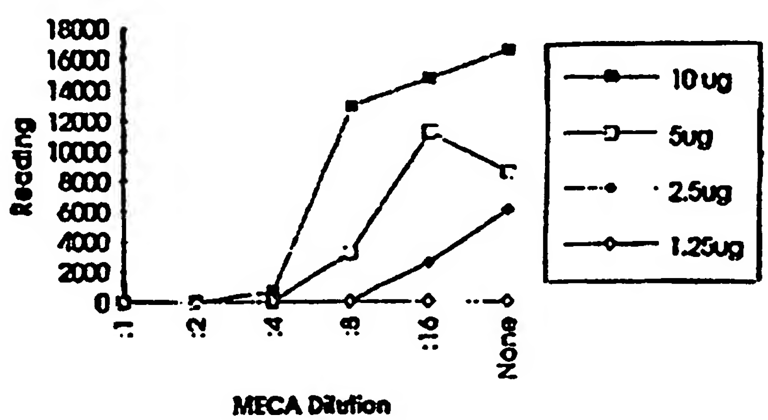
(57) Abstract

The present invention provides novel compounds comprising peptide sequences which mimic the conserved amino acid motif LDTSL of MAdCAM-1 and which have groups bonded to the N- and C-termini. Also provided are methods of inhibiting the interaction of a cell bearing a ligand of MAdCAM-1, such as human $\alpha4\beta7$, with MAdCAM-1 or a portion thereof (e.g., the extracellular domain), comprising contacting the cell with a compound of the present invention.



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INHIBITORS OF MAdCAM-1-MEDIATED INTERACTIONS AND METHODS OF USE THEREFOR

Description

Related Applications

This application is a Continuation-In-Part of U.S. Serial No. 08/582,740, filed January 4, 1996, the teachings of which are incorporated herein by reference in their entirety.

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Background of the Invention

Lymphocyte homing from the circulation to the lymphoid tissues and migration to sites of inflammation is regulated by interaction with receptors expressed in postcapillary venules, including high endothelial venules (HEV) found in secondary lymphoid tissues (e.g., mesenteric lymph nodes, Peyer's Patches (PP)) (Bevilacqua, M.P., *Annu. Rev. Immunol.*, 11:767-804 (1993); Butcher, E.C., *Cell*, 67: 1033-1036 (1991); Picker, L.J., *et al.*, *Annu. Rev. Immunol.*, 10:561-591 (1992); and Springer, T.A., *Cell*, 76: 301-314 (1994)). These interactions are tissue specific in nature.

Inflammation (e.g., chronic inflammation) is characterized by infiltration of the affected tissue by leukocytes, such as lymphocytes, lymphoblasts, and

mononuclear phagocytes. The remarkable selectivity by which leukocytes preferentially migrate to various tissues during both normal circulation and inflammation results from a series of adhesive and activating events involving multiple receptor-ligand interactions as proposed by Butcher and others (Butcher, E.C., *Cell*, 67: 1033-1036 (1991); vonAdrian, U.H., *et al.*, *Proc. Natl. Acad. Sci. USA*, 88:7538 (1991); Mayadas, T.N., *et al.*, *Cell*, 74:541 (1993); (Springer, T.A., *Cell*, 76:301 (1994)). As an initial step, there is a transient, rolling interaction between leukocytes and endothelium, which results from the interaction of selections (and by α4 integrins in some instances) with their carbohydrate ligands. This interaction, which is characterized by rolling in the direction of flow, can be assessed by known methods (Lawrence, M.B. and T.A. Springer, *Cell*, 65:859 (1991); WO 92/21746, Springer et al., (December 10, 1992)). This is followed by activation events mediated by chemoattractants such as chemokines and their receptors, which cause activation of integrin adhesiveness and influence the direction of migration of leukocytes through the vascular walls. Such secondary signals in turn trigger the firm adhesion of leukocytes to endothelium via leukocyte integrins and their endothelial ligands (Ig-like receptors and the ECM), and subsequent transendothelial migration from the circulation across the vascular endothelium.

In secondary lymphoid tissues, such as Peyer's patches (PPs) and lymph nodes (e.g., peripheral lymph nodes (PLN)), leukocyte trafficking and homing is regulated by interactions of homing receptors on the surface of leukocytes with endothelial cells lining the post-capillary venules, notably high endothelial venules (HEV) (Gowans, J.L. and E.J. Knight, *Proc. R. Soc. Lond.*, 159:257 (1964)).

Receptors termed vascular addressing, which are present on the endothelial cell surface and regulate the migration and subsequent extravasation of lymphocyte subsets. The vascular addressing show restricted patterns of expression and this tissue specific expression makes an important contribution to the specificity of leukocyte trafficking (Picker, L .J. and E.C. Butcher, *Annu. Rev. Immunol.*, 10:561-591 (1992); Berg, E.L., *et al.*, *Cellular and molecular mechanisms of inflammation*, 2:111 (1991); Butcher, E.C., *Cell*, 67:1033-1036 (1991)).

Mucosal vascular addressing MAdCAM-1 (Mucosal Addressing Cell Adhesion Molecule-1) is an immunoglobulin superfamily adhesion receptor for lymphocytes, which is distinct from VCAM-1 and ICAM-1. MAdCAM-1 was identified in the mouse as a ~60 kd glycoprotein which is selectively expressed at sites of lymphocyte extravasation. In particular, MAdCAM-1 expression was reported in vascular endothelial cells of mucosal tissues, including gut-associated tissues or lymph the small and large intestine, and the lactating mammary gland, but not in peripheral lymph nodes. MAdCAM-1 is involved in lymphocyte binding to Peyer's Patches. (Streeter, P.R., *et al.*, *Nature*, 331:41-46 (1988); Nakache, M., *et al.*, *Nature*, 337: 179-181 (1989); Picker, L.J., *et al.*, *Annu. Rev. Immunol.*, 10:561-591 (1992); Briskin, M.J., *et al.*, *Nature*, 363:461 (1993); Berg, E.L., *et al.*, *Nature*, 366:695-698 (1993); Berlin, C., *et al.*, *Cell*, 74:185-195 (1993)). MAdCAM-1 can be induced *in vitro* by proinflammatory stimuli (Sikorski, E.E., *et al.*, *J. Immunol.*, 151:5239-5250 (1993)). cDNA clones encoding murine and primate (e.g., human) MAdCAM-1 have been isolated and sequenced (Briskin, M.J. *et al.*,

Nature, 363: 461-464 (1993); Briskin et al., WO 96/24673, published August 15, 1996; and Briskin, M.J. *et al.*, U.S. Serial No. 08/523,004, filed September 1, 1995, the teachings of each of which is incorporated herein by reference in its entirety).

MAdCAM-1 specifically binds the lymphocyte integrin $\alpha4\beta7$ (also referred to as LPAM-1 (mouse), $\alpha4\beta p$ (mouse)), which is a lymphocyte homing receptor involved in homing to Peyer's patches (Berlin, C., *et al., Cell,* 80:413-422 (1994); Berlin, C., *et al., Cell,* 74:185-195 (1993); and Erle, D.J., *et al., J. Immunol.,* 153: 517-528 (1994)). In contrast to VCAM-1 and fibronectin, which interact with both $\alpha4\beta1$ and $\alpha4\beta7$ (Berlin, C., *et al., Cell,* 74: 185-195 (1993); Strauch, U.S., *et al., Int. Immunol.,* 6:263 (1994)), MAdCAM-1 is a selective ligand for $\alpha4\beta7$ receptor.

Inflammatory bowel disease (IBD), such as ulcerative colitis and Crohn's disease, for example, can be a debilitating and progressive disease involving inflammation of the gastrointestinal tract. Affecting an estimated two million people in the United States alone, symptoms include abdominal pain, cramping, diarrhea and rectal bleeding. IBD treatments have included anti-inflammatory drugs (such as corticosteroids and sulfasalazine), immunosuppressive drugs (such as 6-mercaptopurine, cyclosporine and azathioprine) and surgery (such as, colectomy). Podolsky, *New Engl. J. Med.,* 325:928-937 (1991) and Podolsky, *New Engl. J. Med.,* 325:1008-1016 (1991). There is a need for inhibitors of MAdCAM-1 function to provide new therapies useful in the treatment of IBD and other diseases involving leukocyte infiltration of the gastrointestinal tract or other mucosal tissues.

Summary of the Invention

As shown herein, the conserved amino acid motif LDTSL (SEQ ID NO:1) is involved in Mucosal Addressing Cell Adhesion Molecule-1 (hereinafter "MAdCAM-1") binding to MAdCAM-1 ligands, such as human $\alpha4\beta7$. In addition, compounds containing this peptide sequence or truncated versions thereof, e.g., Asp-Thr and Leu-Asp-Thr, bind to $\alpha4\beta7$ and can inhibit adhesion of leukocytes expressing $\alpha4\beta7$ on the cell surface to MAdCAM-1. It has also been discovered that groups on the N- and C-termini of the peptide sequences enhance the binding of these compounds to $\alpha4\beta7$ and are more potent inhibitors of interaction between MAdCAM-1 and its ligands. Accordingly, the present invention provides novel compounds comprising peptide sequences which mimic the conserved amino acid motif LDTSL and which have groups bonded to the N- and C-termini.

Also provided are methods of inhibiting the interaction of a cell bearing a ligand of MAdCAM-1, including $\alpha4\beta7$ integrins, with MAdCAM-1 or a portion thereof (e.g., the extracellular domain), comprising contacting the cell with a compound of the present invention. In one embodiment, the invention relates to a method of inhibiting the MAdCAM-mediated interaction of a first cell bearing an $\alpha4\beta7$ integrin with MAdCAM, for example with a second cell bearing MAdCAM, comprising contacting the first cell with a compound of the present invention. In another embodiment, the invention relates to a method of treating an individual suffering from a disease associated with leukocyte recruitment to tissues (e.g., endothelium) expressing the molecule MAdCAM-1.

One embodiment of the present invention is a method of inhibiting the binding of a cell such as a leukocyte expressing a ligand for MAdCAM-1 on the cell surface (e.g., $\alpha4\beta7$) to MAdCAM-1, for example to endothelial cells expressing MAdCAM-1 on the cell surface. The method

comprises contacting the leukocytes with an effective amount of an inhibitor represented by Structural Formula (I):

$$R^1\text{-X-Y-Z-}R^2 \qquad \text{(I)}$$

Y is a pentapeptide $[AA]_1$-$[AA]_2$-$[AA]_3$-$[AA]_4$-$[AA]_5$.

$[AA]_1$ is selected from the group consisting of leucine, valine, isoleucine, alanine, phenylalanine, glycine, N-methylleucine, serine, threonine, ornithine, cysteine, aspartic acid, glutamic acid and lysine.

$[AA]_2$ is selected from the group consisting of aspartic acid, glutamic acid, phenylalanine and tyrosine.

$[AA]_3$ is selected from the group consisting of threonine, serine, valine, proline and 4-hydroxyproline.

$[AA]_4$ is selected from the group consisting of serine, cysteine, aspartic acid, glutamic acid, proline, 4-hydroxyproline, threonine, valine, isoleucine, alanine, glycine, ornithine and lysine.

$[AA]_5$ is selected from the group consisting of leucine, valine, isoleucine, N-methylleucine, threonine, ornithine, serine, alanine, glycine, phenylalanine, cysteine, aspartic acid, glutamic acid and lysine.

X and Z are independently chosen from the group consisting of a covalent bond, an amino acid or a peptide. Each amino acid in X and Z is independently selected from the group of naturally occurring amino acids. X and Z are preferably covalent bonds.

R^1 is R^3-CO-.

R^2 is -NR^4R^5.

R^3 is selected from the group consisting of a lower alkyl group, a substituted lower alkyl group, an aryl group, a substituted aryl group, a heteroaryl group and a substituted heteroaryl group.

R^4 and R^5 are each independently selected from the group consisting of hydrogen, a lower alkyl group, a substituted lower alkyl group, an aryl group, a substituted aryl group, a heteroaryl group and a substituted heteroaryl group. R^4 and R^5 are not both -H. Taken together, R^4 and R^5 can also form a heterocyclic ring.

Taken together, X, Y and Z form a peptide containing no more than about fifteen amino acid residues.

The compound represented by Structural Formula (I) is a peptide having a group bonded to the nitrogen atom at the N-terminus and a second group bonded to the carbonyl at the C-terminus. In one aspect, the peptide is linear. The N-terminus of Y is not bonded to arginine or an arginine derivative.

Optionally, the peptide formed by X, Y and Z is cyclized to form a ring. When cyclized, the ring can be formed by an amide linkage, an ester linkage or a disulfide linkage between two amino acids in the peptide. When the ring is formed by an amide linkage between the N-terminus and C-terminus of the peptide, R^1 and R^2 together form a covalent bond between the carbonyl at the C-terminus of the peptide and the nitrogen at the N-terminus of the peptide.

In another embodiment of the method of inhibiting the binding of a cell such as a leukocyte expressing a ligand for MAdCAM-1 on the cell surface (e.g., $\alpha4\beta7$) to MAdCAM-1, for example endothelium expressing the molecule MAdCAM-1, the inhibitor administered is represented by Structural Formula (II):

$$R^1\text{-}X\text{-}Y'\text{-}Z\text{-}R^2 \qquad \text{(II)}$$

R^1, R^2, X and Z are as defined for Structural Formula (I). Y' represents a dipeptide having the sequence Asp-Thr, a tripeptide having the sequence Leu-Asp-Thr, or a pentapeptide $[AA]_1$-$[AA]_2$-$[AA]_3$-$[AA]_4$-$[AA]_5$ having the

sequence Leu-Asp-Thr-Ser-Leu (SEQ ID NO: 1) with the proviso that any single one of $[AA]_1$, $[AA]_2$, $[AA]_3$, $[AA]_4$ or $[AA]_5$ can vary, being any naturally occurring amino acid. The nitrogen at the N-terminus of Y' may be bonded to any naturally occurring amino acid (including arginine) with the proviso that the N-terminus of Y' may not be bonded to glycine or sarcosine when Y' is Asp-Thr and the peptide formed from X-Y'-Z is cyclized, as described below.

X, Y' and Z taken together form a peptide containing no more than about fifteen amino acids. In one aspect, the peptide formed by X, Y' and Z is linear. Preferably X and Z are each a covalent bond. Optionally, the peptide formed by X, Y' and Z is cyclized to form a ring. When cyclized, the ring can be formed by an amide linkage, an ester linkage or a disulfide linkage between two amino acids in the peptide. When the ring is formed by an amide linkage between the N-terminus and C-terminus of the peptide, R^1 and R^2 together form a covalent bond between the carbonyl at the C-terminus of the peptide and the nitrogen at the N-terminus of the peptide.

Another embodiment of the present invention is a novel compound. The compound is represented by Structural Formula II, as described above.

Another embodiment, of the present invention is a method of treating an individual suffering from a disease associated with leukocyte infiltration of tissues expressing the molecule MAdCAM-1. The method comprises administering to the individual a therapeutically effective amount of an inhibitor represented by Structure Formula (I) or an inhibitor represented by Structural Formula (II).

Compounds of the present invention are inhibitors of the binding of MAdCAM-1 to the receptor α4β7 and are therefore useful in the treatment of diseases such as inflammatory bowel disease with the potential for fewer

side effects in other tissues where adhesion is mediated by α4β1 integrin, for example.

The compounds of the present invention are also useful in diagnostic and research applications. For example, the compounds can be used as immunogens (e.g., when conjugated to a suitable carrier) to induce the formation of antibodies which selectively bind MAdCAM-1 or a portion thereof. These antibodies can in turn be used to identify cells expressing MAdCAM-1 on their cell surface or detect MAdCAM-1 in a sample. In addition, the compounds of the present invention can be labelled and used to detect α4β7 integrin and/or quantitate expression of $\mu_4\beta_7$ integrin on the surface of cells.

Brief Description of the Drawings

Figure 1 is a graph illustrating a titration experiment used to select the amount of rat anti-murine κ capture antibody which was used in adhesion assays. MAdCAM-mCκ fusion protein was bound to the surface of a well via various amounts of capture antibody, and the adhesion of fluorescently labelled HUT 78 cells to fusion protein in the presence of increasing amounts of anti-MAdCAM-1 antibody was monitored. Reading (Y-axis) is in arbitrary fluorescence units. 50 μl of a 10 μg/ml (■), 5 μg/ml (□), 2.5 μg/ml (♦) or 1.25 μg/ml (◊) solution of purified rat anti-murine κ antibody was used. Anti-murine MAdCAM-1 antibody MECA-367 was used as neat supernatant or diluted 1:2, 1:4, 1:8 or 1:16 (indicated on the X-axis by :1 (neat), :2, :4, :8, and :16, respectively). "None" indicates no MECA-367 antibody (buffer control) was present.

Figure 2 is a graph illustrating the inhibition by compound 793-1a of the adherence of fluorescently labelled cells bearing α4β7 (HUT 78 human T cell lymphoma cells) to a MAdCAM-mCκ fusion protein bound by capture antibody. The

various concentrations (μM) of compound used were plotted against a ratio (calculated from measurements of adherent fluorescent cells; see Example 2).

Figure 3 is a schematic illustration of a MAdCAM-1/mCκ fusion gene and encoded fusion protein. A BamHI site at the junction of the MAdCAM-1 and mCκ sequences introduces codons for Gly-Ser (SEQ ID NO: 63 and SEQ ID NO: 64). The fusion gene encodes the signal peptide (SEQ ID NO: 65 and SEQ ID NO: 66, partial signal peptide) and complete extracellular domain of MAdCAM-1 through the threonine residue at position 365. Single letter amino acid codes are used.

Figure 4 is an illustration of the nucleotide sequence determined from subclones of cDNA clone 4 encoding human MAdCAM-1 (SEQ ID NO: 67), and the sequence of the predicted protein encoded by the open reading frame (MAdCAM-1) (SEQ ID NO: 68). The predicted signal peptide and transmembrane region are underlined in bold. Cysteine residues of the two Ig-like domains are boxed, as are potential N-linked glycosylation sites. The mucin domain consisting of 71 amino acids is outlined by a thin bold line. The LDTSL (SEQ ID NO: 1) motif begins at amino acid 63.

Figure 5 is an illustration of the nucleotide sequence determined from subclones of cDNA clone 20 encoding human MAdCAM-1 (SEQ ID NO: 69), and the sequence of the predicted protein encoded by the open reading frame (MAdCAM-1) (SEQ ID NO: 70). The predicted signal peptide and transmembrane region are underlined in bold. Cysteine residues of the two Ig-like domains are boxed, as are potential N-linked glycosylation sites. The mucin domain consisting of 47 amino acids is outlined by a thin bold line. The LDTSL (SEQ ID NO: 1) motif begins at amino acid 63. The two human cDNA clones are probably isoforms encoded by genomic DNA, generated, for example, by alternative splicing or by transcription of two different alleles.

Detailed Description of the Invention

As used herein, "lower alkyl" refers to a hydrocarbon containing from one to about twelve carbon atoms. In one aspect, the term "C3-C12 alkyl" is employed to include all lower alkyls except $-CH_3$ and $-CH_2-CH_3$. The hydrocarbon can be saturated or can contain one or more units of unsaturation. The hydrocarbon can also be branched, straight chained or cyclic.

A "cyclic hydrocarbon" refers to a cycloalkyl group. A "cycloalkyl group" includes carbocyclic rings (e.g., cyclopentyl and cyclohexyl) as well as carbocyclic rings in which one or more carbon atoms is replaced by a heteroatom (e.g., morpholino, piperidinyl, pyrrolidinyl, thiomorpholino or piperazinyl). Also included are cycloalkyl rings fused to other cycloalkyl rings (e.g., decalinyl) or fused to aromatic or heteroaromatic groups (e.g., indanyl, tetralinyl and 9-xanthenyl). A "cyclic hydrocarbon" also includes bridged polycyclic structures such as adamantyl and nonbornyl. As with other lower alkyl groups, a cyclic hydrocarbon can be substituted.

As used herein, "aryl" groups are defined to include aromatic ring systems such as phenyl. "Heteroaryl" groups are defined to include aromatic ring systems which contain heteroatoms, such as 2-furanyl, 3-furanyl, 2-thienyl, 3-thienyl, 2-pyridyl, 2-pyranyl and 3-pyranyl. "Aryl" and "heteroaryl" groups also include fused polycyclic aromatic ring systems in which the aryl or heteroaryl ring is fused to one or more other aryl, heteroaryl or cycloalkyl rings. Examples include α-naphthyl, β-naphthyl, 1-anthracenyl, 2-anthracenyl, 2-benzothienyl, 3-benzothienyl, 2-benzofuranyl, 3-benzofuranyl, 2-indolyl, 2-quinolinyl, 3-quinolinyl, acridinyl and 9-thioxanthanyl. Also included are polycyclic aromatic ring systems in which aryl groups, heteroaryl groups or an aryl and a heteroaryl group are connected by a covalent bond (e.g., 4-phenylphenyl, 3,5-

diphenylphenyl, 4-(2-thienyl)phenyl and 4-(2-furanyl)phenyl)) or a $-(CH)_n-$ bridge (e.g., 4-(phenyl-CH_2)phenyl, 4-(phenylCH_2CH_2-)phenyl, 4-(-CH_2-2-thienyl)phenyl and 4-(-CH_2CH_2-2-thienyl)phenyl), wherein n is an integer from 1 to about 5.

Suitable substituents on a lower alkyl group, an aryl group or a heteroaryl group include C1-C2 alkoxy, ketone, aldehyde, (lower alkyl) ester, aryl, substituted aryl, heteroaryl, substituted heteroaryl benzyl, lower alkyl, fluoro, bromo, iodo, cyano, nitro and the like. A lower alkyl group, an aryl group or a heteroaryl group may have more than one substituent (e.g., 2,2,3,3-tetramethylcyclopropyl, 3,5-diphenylphenyl and 2,4-dichlorophenyl), 2-bromo-4-nitro-pentyl and 2-(3,5-dibromo-benzofuranyl). In addition, a substituted lower alkyl group can have multiple substituents on one carbon atom (e.g., diphenylmethyl, triphenylmethyl, 2,2,3,3-tetramethylcyclopropyl and trifluoromethyl).

R^1 and R^2 are groups covalently bonded to the N-terminal and the C-terminal, respectively, of the peptide sequences of the compounds represented by Structural Formulas (I) and (II).

In one preferred embodiment, R^3 is selected from the group consisting of adamantyl, adamantylmethyl, a C1-C4 alkyl group, a C3-C7 cycloalkyl group, a C3-C7 cycloalkyl group substituted with a C1-C4 alkyl group, an aryl group substituted with a C3-C7 cycloalkyl group, phenyl substituted with a C1-C8 alkyl group, an aryl group, 2-anthracenyl, diphenylmethyl, 1-napthyl, 2-naphthyl, benzyl, indanyl, tetralinyl, triphenylmethyl, triphenyl(C1-C4 alkyl), 9-fluorenyl, styryl, a heteroaryl group, furanyl, thienyl, 9-xanthanyl, 9-thioxanthanyl, acridinyl, pyridyl, quinolinyl, a C1-C4 alkyl group substituted with an aryl group (e.g.,, benzyl and phenylethyl) and a C1-C4 alkyl group substituted with heteroaryl (furanylmethyl,

thienylmethyl, pyridylmethyl, quinolinylmethyl). More preferably, R^3 is selected from the group consisting of triphenylmethyl, diphenylmethyl, 3,5-diphenylphenyl, 2-furanyl, 3-furanyl, 9-xanthenemethyl, 2,2,2-triphenylethyl, 2-anthracene, methyl, cyclopentyl, 2-indolyl, 2-indanyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl, 3-benzothienyl, cyclohexyl, 5-phenylpentyl, 4-isobutyl-α-methylphenylmethyl, 4-biphenylmethyl, α-naphthylmethyl, 4-heptylphenyl, phenylmethyl, *trans* 2-phenylethenyl and 2,2,3,3-tetramethylcyclopropyl.

In another preferred embodiment, R^3 is a substituted or unsubstituted aryl or heteroaryl group. Examples include monocyclic and bicyclic nitrogen-containing heteroaromatic groups, such as a quinolinyl group (e.g., 2-quinolinyl, 2-phenyl-4-quinolinyl, 3-quinolinyl, 4-quinolinyl, 7-quinolinyl), an isoquinolinyl group (e.g., 3-isoquinolinyl), an indolyl group (e.g., 2-indolyl and 5-chloro-2-indolyl), a quinoxalinyl group (e.g., 2-quinoxalinyl) a cinnolinyl group, and a pyrazinyl group. Also included are vinyl groups substituted with substituted or unsubstituted aryl or heteroaryl groups (e.g., *cis* and *trans* styryl and stilbyl, (3-pyridyl)-CH=CH-), polycarbocyclic aromatic hydrocarbons (e.g., 2-naphthyl and 2-anthracyl) and oxygen-containing polycyclic aromatic hydrocarbons (e.g., 9-xanthanyl, 2-benzopyranone, 3-benzopyranone and 2-benzofuranyl). Suitable substituents for an aryl or heteroaryl group are as described above.

R^4 and R^5 are preferably independently selected from the group consisting of a C1-C5 alkyl group, a C1-C5 alkyl group substituted with C1-C4 alkoxy, a C3-C7 cycloalkyl group, a C3-C7 cycloalkyl group substituted with a C1-C5 alkyl group, an aryl group, a substituted C1-C5 alkyl group (e.g.,, suitable substituents include a hydroxyl group, phenyl, substituted phenyl (e.g., suitable substituents

include a C1-C5 alkyl group, C1-C4 alkoxy, halogen, nitro and trifluoromethyl)), a C1-C4 alkyl group substituted with an aryl group (e.g., benzyl, phenylethyl, diphenylethyl, 9-fluorenyl), a heteroaryl group (e.g., benzofuranyl, benzothienyl, furanyl, pyridinyl, quinolinyl, and thienyl) and a C1-C5 alkyl group substituted with a heteroaryl group (e.g., furanylmethyl, thienylmethyl, pyridylmethyl and quinolinylmethyl).

In addition, R^4 and R^5 may, taken together, form a heterocyclic ring including piperidinyl, pyrrolidinyl, morpholino, thiomorpholino or piperazinyl where the N^4-position of the piperazine ring is substituted by a group consisting of hydrogen, acetyl, benzoyl, alkyl, benzyl or phenyl. More preferably, R^4 and R^5 are each independently selected from the group consisting of 2-hydroxyethyl, benzyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl, 3-benzothienyl, $-CH_2$-2-thienyl, $-CH_2$-3-thienyl, $-CH_2$-2-furanyl, $-CH_2$-3-furanyl, 3,4-dimethoxybenzyl, and isopentyl.

Examples of compounds of the present invention include the following:

Cpc-NH-Asp-Thr-N(CH_2CH_2OH)(Benzyl)
Cpc-NH-Asp-Thr-NH-Hexyl
Cpc-NH-Asp-Thr-NH-(3,4-Dimethoxybenzyl)
Cpc-NH-Asp-Thr-NH-CH(Phenyl)$_2$
Idc-NH-Asp-Thr-NH-Benzyl
Chc-NH-Asp-Thr-NH-CH_2Thienyl
Bpc-NH-Asp-Thr-NH-Benzyl
Indc-NH-Asp-Thr-NH-Isopentyl
Bpc-NH-Asp-Thr-NH-CH_2Thienyl

Abbreviations are defined in Table 3.

In a preferred embodiment, R^3 is selected from the group consisting of diphenylmethyl, triphenylmethyl, *trans* 2-phenyl-ethylenyl, 2-phenyl-ethynyl, 2-benzofuranyl,

3-benzofuranyl, 2-benzothienyl and 3-benzothienyl, R^4 is selected from the group consisting of 2-hydroxyethyl, benzyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl, 3-benzothienyl, $-CH_2$-2-thienyl, $-CH_2$-3-thienyl, $-CH_2$-2-furanyl, $-CH_2$-3-furanyl and R^5 is -H.

As used herein, "[AA]" represents an amino acid and "$[AA]_1 \cdots [AA]_n$" represents a peptide, wherein $[AA]_1$ is the N-terminal amino acid and $[AA]_n$ is the C-terminal amino acid. Unless otherwise indicated, amino acids in the peptides are presented from left to right, with the left end being the N-terminus and the right end being the C-terminus. The amino acids within the peptide can be similarly designated. For example, "$[AA]_2$" and "$[AA]_{n-1}$" refer to the second amino acid from the N-terminal and C-terminal, respectively. This nomenclature permits each amino acid of X and Z to be designated. For example, "$[AA]_1$ of Z" refers to the amino acid at the N-terminus of Z when Z is a peptide. "$[AA]_2$ of X" refers to the second amino acid from the N-terminus of X when X is a peptide. "$[AA]_{n-1}$ of X" refers to the second amino acid from the C-terminus of X when X is a peptide. It is to be understood that the amino acids in Y are numbered in sequence from 1-5 (Y' is numbered in sequence from 1-5, 1-3 or 1-2 when Y' is a pentapeptide, a tripeptide or a dipeptide, respectively) beginning at the N-terminus and ending at the C-terminus. Thus, "$[AA]_2$ of Y" refers to the second amino acid from the N-terminal of Y. It is also to be understood that "$[AA]_1$ of X" (or "$[AA]_1$ of Z") can be used to represent X (or Z) when X (or Z) is a single amino acid.

In one embodiment Y is Y', e.g., Asp-Thr, Leu-Asp-Thr or a pentapeptide $[AA]_1$-$[AA]_2$-$[AA]_3$-$[AA]_4$-$[AA]_5$ having the sequence Leu-Asp-Thr-Ser-Leu (SEQ ID NO: 1) with the proviso that a single one of $[AA]_1$, $[AA]_2$, $[AA]_3$, $[AA]_4$ or $[AA]_5$ can vary, being any naturally occurring amino acid. A "naturally occurring amino acid" is an amino acid that

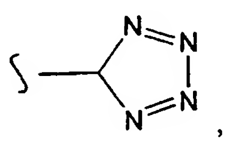
occurs in nature. "Naturally occurring amino acids" includes, but are not limited to serine, threonine, cysteine, glycine, valine, alanine, leucine, isoleucine, aspartic acid, glutamic acid, glutamine, asparagine, lysine, arginine, ornithine, tyrosine, phenylalanine, histidine, proline, 4-hydroxyproline, tryptophan and methionine.

For Structural Formula (I), if X is an amino acid, X may not be arginine or a derivative of arginine. In addition, for Structural Formula (I) if X is a peptide, the amino acid at the C-terminus of X may not be arginine or an arginine derivative. Arginine derivatives include N and/or N' C1-C4 alkylated arginines (e.g., N-alkyl arginine, N,N-dialkyl arginine, N,N'-dialkyl arginine, and N,N,N'-trialkyl arginine). Arginine derivatives also include arginine mimics (e.g., p-aminomethyl arginine), arginine isomers (e.g., norarginine), arginine having substituents in the side chain (e.g., nitro, halo or C1-C4 alkyl) and arginines containing one or more additional carbon atoms in the side chain (e.g., homoarginine).

In a preferred embodiment, Y' is the tripeptide Leu-Asp-Thr or the dipeptide Asp-Thr, and examples of preferred groups for R^3 include substituted and unsubstituted phenyl (e.g., -2,5-diCF_3, 2-CF_3, 3-CF_3, 1,2-difluoro, 3,5-difluoro, 2,5,6-trifluoro, 3-(*n*-hexyloxy), 3-chloro, 4-*t*-butyl and 4-phenyl substituted phenyl), thienyl (e.g., 2-thienyl and 3-halo-2-thienyl), indolyl (e.g., 2-indolyl), pyrimidyl (e.g., 1-chlor-3-trifluoro-4-pyrimidyl), pyridyl (e.g., 2-methyl-6-chloro-3-pyridyl), benzofuranyl (e.g., 2-benzofuranyl), isoquinlinyl (e.g., 3-isoquinolinyl), 3-isoquinolinyl-CO-NH-$(CH_2)_x$- (wherein x is an integer from 1-4) and benzopyranone groups (e.g., 2- and 3-benzopyranone). R^3 is more preferably 3-isoquinolinyl or 2-benzofuranyl, R^4 is preferably -H and R^5 is preferably

benzyl, substituted benzyl (e.g., 3-CF_3, 3-methyl, 4-CH_3, 2-CH_3, 4-fluoro, 3,4-difluoro, 2,5-difluoro, 3,4-methylenedioxy, 4-*N*-morpholino and 4-OCH_3 substituted benzyl), phenethyl, substituted phenethyl (e.g., 3,4-dimethoxy and 4-SO_2NH_2 substituted phenethyl), phenpropyl, substituted phenpropyl, heteroaryl-CH_2- (wherein heteroaryl is, e.g., 3-furanyl, 3-thienyl and 4-pyridinyl), substituted heteroaryl-CH_2- (e.g., 6-(2-methyl)-quinolinyl), lower alkyl, substituted lower alkyl (e.g., isopropyl, methoxymethyl, 2-hydroxyethyl, 3-hydroxypropyl, 3-(2-methyl-*N*-piperidinyl)propyl, 1-(ethyl)-1-propyl, 2-(1-cyclohexene)ethyl and 1-(cyclohexyl)ethyl) and cycloalkyl (e.g., cyclopropyl, cyclopentyl, cyclohexyl and cyclooctyl). Other examples of R^5 are represented by the following structural formulas:

(III)



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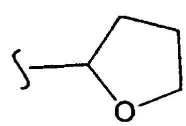

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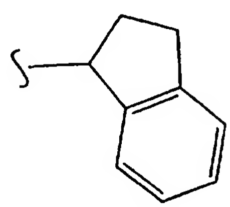
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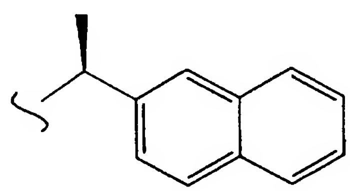
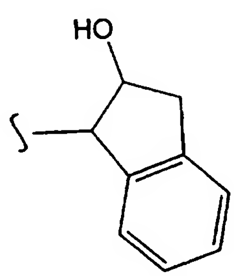


and



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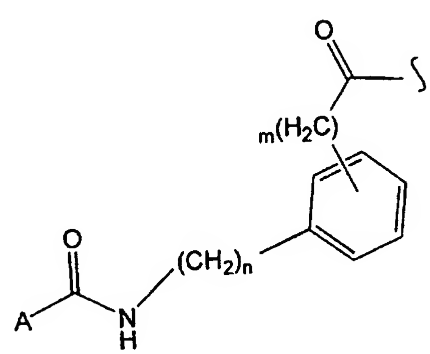
In addition, taken together, R_4 and R_5 can form a heterocyclic ring such as pyrrolidinyl, substituted pyrrolidinyl (e.g., 3,3-dimethyl substituted and 3-dimethylamino substituted pyrrolidinyl), indoline, isomers of indoline, substituted indoline, substituted isomers of indoline, tetrahydroisoquinoline, substituted tetrahydroisoquinoline, tetrahydroquinoline, substituted tetrahydroquinoline, piperidones (e.g., 4-piperidone), substituted piperidones, piperidine, substituted piperidines, tetrahydro-oxazines (e.g., 1,3-tetrhydro-oxazine and morpholine) and substituted tetrahydro-oxazines (e.g., 2,4-dimethyl-1,3-tetrahydro-oxazine and 3,5-dimethyl morpholine).

In another embodiment, when Y' is the tripeptide Leu-Asp-Thr or the dipeptide Asp-Thr, R^1 is represented by Structural Formula (IV):



(IV)

A is an aryl group, a substituted aryl group a heteroaryl group or a substituted heteroaryl group, as described above. Examples include 3-isoquinolinyl, 2-

indolyl, 5-chloro-2-indolyl, 2-benzofuranyl, phenyl and *trans*-styryl.

n and m are each independently zero or one. Preferably, n+m=1.

The substitution pattern on the phenyl ring in Structural Formula (IV) can be *ortho*, *meta* or *para*.

In another preferred embodiment Y or Y' is the pentapeptide $[AA]_1$-$[AA]_2$-$[AA]_3$-$[AA]_4$-$[AA]_5$] wherein:

amino acid $[AA]_1$ is selected from the group consisting of leucine, isoleucine, alanine, valine, glycine, phenylalanine and N-methylleucine;

amino acid $[AA]_2$ is selected from the group consisting of aspartic acid, glutamic acid, phenylalanine and tyrosine;

amino acid $[AA]_3$ is selected from the group consisting of threonine, serine, valine, proline and 4-hydroxyproline;

amino acid $[AA]_4$ is selected from the group consisting of serine, cysteine and threonine; and

amino acid $[AA]_5$ is selected from the group consisting of leucine, alanine, valine, isoleucine, alanine, glycine, phenylalanine and N-methylleucine.

In another aspect, any one or more of $[AA]_1$, $[AA]_2$, $[AA]_3$, $[AA]_4$ and $[AA]_5$ in the pentapeptide of Y or Y' can also be a non-naturally occurring amino acid. $[AA]_1$ and/or $[AA]_5$ can be a non-naturally occurring amino acid in which the side chain is a C2-C7 substituted or unsubstituted alkyl group or a substituted phenyl group. Side chain alkyl groups can be straight chained, branched or cyclic. Suitable substituents for an alkyl side chain include non-polar groups such as C1-C2 alkyl, halo, cyano, C1-C3 alkoxy, phenyl or substituted phenyl. Suitable substituents for a side chain phenyl group include non-polar groups such as C1-C2 alkyl, halo, cyano or -C1-C3

alkoxy. $[AA]_2$ can be a non-naturally occurring amino acid having a C3-C6 straight chained or branched alkyl group substituted with a carboxylic acid. $[AA]_3$ and/or $[AA]_4$ can be a non-naturally occurring amino acid in which the side chain is a C2-C6 straight chained or branched alkyl group substituted with an alcohol or thiol.

The compounds represented by Structural Formulas (I) and (II) are peptides X-Y-Z or X-Y'-Z, respectively, with groups attached to the N-terminus and C-terminus. Preferably, X and Z are covalent bonds, i.e. the compounds represented by Structural Formulas (I) and (II) are peptides Y or Y', respectively, with group R^1 attached to the N-terminal of Y or Y', and with group R^2 attached to the C-terminal of Y or Y'. In one aspect, X-Y-Z or X-Y'-Z is a linear peptide. In another aspect, X-Y-Z or X-Y'-Z is cyclized.

As used herein, "cyclized" refers to forming a ring by a covalent bond between suitable side chains of two amino acids in the peptide. For example, a disulfide can be formed between the sulfur atoms in the side chains of two cysteines. Alternatively, an ester can be formed between the carbonyl carbon in the side chain of, for example, glutamic acid or aspartic acid, and the oxygen atom in the side chain of, for example, serine or threonine. An amide can be formed between the carbonyl carbon in the side chain of, for example, glutamic acid or aspartic acid, and the amino nitrogen in side chain of, for example, lysine or ornithine.

"Cyclized" also refers to forming a ring by a peptide bond between the nitrogen atom at the N-terminus and the carbonyl carbon at the C-terminus. In this case, R^1 and R^2 together form a covalent bond.

"Cyclized" also refers to forming a ring by forming a covalent bond between the nitrogen at the N-terminus of the compound and the side chain of a suitable amino acid in the

peptide. For example, an amide can be formed between the nitrogen atom at the N-terminus and the carbonyl carbon in the side chain of aspartic acid or glutamic acid. Alternatively, the compounds represented by Structural Formulas (I) and (II) can also be cyclized by forming a covalent bond between the carbonyl at the C-terminus of the compound and the side chain of a suitable amino acid in the peptide. For example, an amide can be formed between the carbonyl carbon at the C-terminus and the amino nitrogen atom in the side chain of lysine or ornithine; an ester can be formed between the carbonyl carbon at the C-terminus and the hydroxyl oxygen atom in the side chain of serine or threonine.

Preferably, the ring of the cyclized compounds of the present invention contains four to nine amino acids. The peptide is preferably cyclized to maximize within the ring the number of amino acids involved in the binding of the peptide to a ligand of MAdCAM-1, such as human $\alpha4\beta7$. Thus, the ring contains at least four of the five amino acids in Y or Y', when Y or Y' is a pentapeptide. More preferably, the ring contains all of the amino acids in Y or Y'.

For compounds represented by Structural Formula (I), the ring can be formed by a bond between the side chain of an amino acid selected from the group consisting of $[AA]_{n-3}$ of X, $[AA]_{n-2}$ of X, $[AA]_{n-1}$ of X, $[AA]_n$ of X (if the specific amino acid is present in X) and $[AA]_1$ of Y and the side chain of any one of the amino acids selected from the group consisting of $[AA]_5$ of Y, $[AA]_1$ of Z, $[AA]_2$ of Z, $[AA]_3$ of Z and $[AA]_4$ of Z (if the specific amino acid is present in (X and/or Z), with the proviso that the ring contains from four to nine amino acids. The ring can also be formed by a bond between the side chain of $[AA]_4$ and the side chains of any one of $[AA]_{n-4}$ of X, $[AA]_{n-3}$ of X, $[AA]_{n-2}$ of X, $[AA]_{n-1}$ of X, $[AA]_n$ of X (if the specific amino acid is present in X) or $[AA]_1$ of Y when the ring contains only four of the five

amino acids of Y. Alternatively, the ring can also be formed by a bond between the side chain of $[AA]_2$ and the side chains of any one of $[AA]_5$ of Y, $[AA]_1$ of Z, $[AA]_2$ of Z, $[AA]_3$ of Z, $[AA]_4$ of Z or $[AA]_5$ of Z (if the specific amino acid is present in Z) when the ring contains only four of the five amino acids of Y.

For compounds represented by Structural Formula (II), when Y' is a pentapeptide, the ring can be formed as described in the preceding paragraph. When Y' is a tripeptide, the ring can be formed by a bond between the side chain functional group of an amino acid selected from the group consisting of $[AA]_{n-5}$ of X, $[AA]_{n-4}$ of X, $[AA]_{n-3}$ of X, $[AA]_{n-2}$ of X, $[AA]_{n-1}$ of X, $[AA]_n$ of X and $[AA]_1$ of Y' and the side chain functional group of any one of the amino acids selected from the group consisting of $[AA]_3$ of Y', $[AA]_1$ of Z, $[AA]_2$ of Z, $[AA]_3$ of Z, $[AA]_4$ of Z, $[AA]_5$ of Z and $[AA]_6$ of Z (if the specific amino acid is present in X and/or Z), with the proviso that the ring contains from four to nine amino acids. When Y' is a dipeptide, the ring can be formed by a bond between the side chain of an amino acid selected from the group consisting of $[AA]_2$ of Y^1, $[AA]_{n-5}$ of X, $[AA]_{n-4}$ of X, $[AA]_{n-3}$ of X, $[AA]_{n-2}$ of X, $[AA]_{n-1}$ of X, $[AA]_n$ of X and $[AA]_1$ of Y' and the side chain functional group of any one of the amino acids selected from the group consisting of $[AA]_2$ of Y', $[AA]_1$ of Z, $[AA]_2$ of Z, $[AA]_3$ of Z, $[AA]_4$ of Z, $[AA]_5$ of Z, $[AA]_6$ of Z and $[AA]_7$ of Z (if the specific amino acid is present in X and/or Z), with the proviso that the ring contains from four to nine amino acids.

When the ring contains all of the amino acids of Y or Y', the compound of Structural Formula I or II can be cyclized by a peptide bond between the nitrogen at the N-terminus of X and the carbonyl carbon at the C-terminal of Z.

In one embodiment, X is an amino acid or has an amino acid sequence which is the same as that immediately N-terminal to the LDTSL (SEQ ID NO: 1) motif of human MAdCAM-1, and Z is an amino acid or has an amino acid sequence which is the same as that immediately C-terminal to the LDTSL (SEQ ID NO: 1) motif of human MadCAM-1. In a further aspect, any one or two amino acids in X and Z can replaced by a naturally occurring amino acid or a non-naturally occurring amino acid, as described above.

Peptide sequences in the compounds of the present invention may be synthesized by solid phase peptide synthesis (e.g., BOC or FMOC) method, by solution phase synthesis, or by other suitable techniques including combinations of the foregoing methods. The BOC and FMOC methods, which are established and widely used, are described in Merrifield, *J. Am. Chem. Soc. 88*:2149 (1963); Meienhofer, *Hormonal Proteins and Peptides*, C.H. Li, Ed., Academic Press, 1983, pp. 48-267; and Barany and Merrifield, in *The Peptides*, E. Gross and J. Meienhofer, Eds., Academic Press, New York, 1980, pp. 3-285. Methods of solid phase peptide synthesis are described in Merrifield, R.B., *Science*, *232*: 341 (1986); Carpino, L.A. and Han, G.Y., *J. Org. Chem.*, *37*: 3404 (1972); and Gauspohl, H. *et al.*, *Synthesis*, *5*: 315 (1992)). The teachings of these six articles are incorporated herein by reference in their entirety. Examples of the synthesis of the compounds having the structure represented by Structural Formulas (I) and (II) are disclosed in Examples 1, General Procedures A-C.

N- and C-terminal modified peptides can be prepared on an oxime resin using a modification of procedures described in DeGrado and Kaiser, *J. Org. Chem. 47*:3258 (1982), the teachings of which are incorporated herein by reference.

Oxime resin can be obtained from Nova Biochem, Inc. The resin is acylated with, for example, Fmoc-Thr(*t*-Bu)-OH (4-6 equivalents), HBTU (4-6 equivalents)/NMM (8-12 equivalents) in dimethylformamide (DMF) at room temperature over about twenty hours. The resin is then washed with DMF, followed by methylene chloride and dried under vacuum prior to use. A peptide can then be synthesized using the Fmoc-Thr(*t*-Bu)-Oxime resin and General Procedure C in Example 1. The resulting peptide is reacted with the desired amine (8-12 equivalents) in methylene chloride for twelve hours. The resin is then washed with methylene chloride. The filtrate is washed with 5% citric acid in water and dried over anydrous magnesium sulfate. The solvent is removed and the resulting residue dried under vacuum to give the desired N- and C-terminal modified residue.

Methods of cyclizing compounds having peptide sequences are described, for example, in Lobl *et al.*, WO 92/00995, the teachings of which are incorporated herein by reference in their entirety. Cyclized compounds can be prepared by protecting the side chains of the two amino acids to be used in the ring closure with groups that can be selectively removed while all other side-chain protecting groups remain intact. Selective deprotection is best achieved by using orthogonal side-chain protection such as allyl (OAI) (for the carboxyl group in the side chain of glutamic acid or aspartic acid, for example), allyloxy carbonyl (Aloc) (for the amino nitrogen in the side chain of lysine or ornithine, for example) or acetamidomethyl (Acm) (for the sulfhydryl of cysteine) protecting groups. OAI and Aloc are easily removed by Pd° and Acm is easily removed by iodine treatment. Examples of cyclizing a compound represented by Structural Formulas (I) and (II) by forming a disulfide bond are given in Example 1 and General Procedure D.

In another example, the cyclic peptide K*LDTSLD* (SEQ ID NO: 2), cyclized between the side chains of lysine and the N-terminal aspartic acid ("*" indicates a cyclizing amino acid) peptide can assembled on PAL-PEG-PS-resin using Nα-Fmoc-amino acids and t-butyl side-chain protection as described in General Procedures A and B in Example 1, except D* and K* is incorporated in the linear chain as Fmoc-Asp(OAI)-OH and Fmoc-Lys(Aloc)-OH, respectively. Allyl functions are removed by treatment with $Pd(PPH_3)_4$, morpholine and triphenylphosphine in dry THF at room temperature and cyclization is achieved with PYBOP. Finally the peptide is deblocked and removed from the resin as described in General Procedures A and B.

In yet another example, the head to tail cyclic peptide TSLLD (SEQ ID NO: 62) can be assembled on Fmoc-Asp(OPAC-resin)-OAI using Fmoc-amino acids and t-butyl side-chain protection, as described in General Procedures A and B in Example 1. The allyl function is removed by treatment with $Pd(PPh_3)_4$, morpholine and triphenylphosphine in dry THF at room temperature and cyclization is achieved with PYBOP. Finally the peptide is deblocked and removed from the resin as described in General Procedures A and B to give cyclic LDTSL peptide. Kates, S. A., *et al.*, "Peptides: Design, Synthesis and Biological Activity," Basava C, Anantharamalah GM, eds., pp. 39-58 Boston: Birkhauser.

<u>Methods</u>

Peptides which mimic the conserved amino acid motif LDTSL (SEQ ID NO: 1) and which are modified at the N- and C-termini, including the compounds of the present invention, are useful in a method of inhibiting (e.g., reducing or preventing) the binding of a cell bearing a ligand of MAdCAM-1 on the cell surface to MAdCAM-1 or a portion thereof (e.g., the extracellular domain).

According to the method, the cell bearing a ligand for MAdCAM-1 is contacted with an effective amount of an (i.e., one or more) inhibitor (as represented by Structural Formula (I) or (II)). As used herein, an inhibitor is a compound which inhibits (reduces or prevents) the binding of MAdCAM-1 to a ligand, including α4β7 integrin, and/or which inhibits the triggering of a cellular response mediated by the ligand. An effective amount can be an inhibitory amount (such as an amount sufficient to achieve inhibition of adhesion of a cell bearing a MAdCAM-1 ligand to MAdCAM-1). Ligands for MAdCAM-1 include α4β7 integrins, such as human α4β7 integrin, and its homologs from other species such as mice (also referred to as α4βp or LPAM-1 in mice).

For example, the adhesion of a cell which naturally expresses a ligand for MAdCAM-1, such as a leukocyte (e.g., B lymphocyte, T lymphocyte) or other cell which expresses a ligand for MAdCAM-1 (e.g., a recombinant cell), to MAdCAM-1 can be inhibited *in vivo* or *in vitro* according to the method. In one embodiment, the MAdCAM-mediated interaction of a first cell bearing a ligand for MAdCAM-1 with a second cell bearing MAdCAM-1 is inhibited by contacting the first cell with an inhibitor according to the method.

The adhesion of cells to MAdCAM-1 or a suitable portion thereof can be inhibited. For example, MAdCAM-1 or a suitable portion thereof can be a soluble protein or can be expressed on the surface of a suitable cell, such as a cell which naturally expresses MAdCAM-1 (e.g., an endothelial cell), a suitable cell line, or other cell which expresses MAdCAM-1 (e.g., a recombinant cell). Suitable portions of MAdCAM-1 include, for example, a portion comprising the LDTSL (SEQ ID NO: 1) motif capable of mediating adhesion (e.g., a portion comprising the entire extracellular domain, or both N-terminal immunoglobulin-like domains)

(see e.g., Briskin, *et al.*, WO 96/24673, published August 15, 1996). MAdCAM-1 or a portion thereof can be part of a larger molecule, such as a fusion protein. For example, a soluble hybrid protein comprising a mammalian (e.g., human or other primate, murine) MAdCAM-1 moiety fused at its C-terminus, to the N-terminus of an immunoglobulin moiety (e.g., one or more immunoglobulin constant regions) to obtain an immunoadhesin, such as those prepared according to Capon *et al.* (U.S. Patent No. 5,428,130), can be used. For example, as shown herein, the adhesion of a T cell lymphoma line which expresses $\alpha4\beta7$ to a fusion protein comprising the extracellular domain of murine MAdCAM-1 joined to the constant region of a murine κ light chain was inhibited according to the method (See e.g., Example 2 and Table 2). The use of a fusion protein comprising the extracellular domain of human MadCAM-1 fused to a human δ_1 constant regions is described in Example 3. These or other recombinant soluble receptor molecules are useful in the method.

In another aspect, the invention relates to a method of treating an individual (e.g., a mammal, such as a human or other primate) suffering from a disease associated with leukocyte (e.g., lymphocyte, monocyte) infiltration of tissues (including recruitment and/or accumulation of leukocytes in tissues) which express the molecule MAdCAM-1. The method comprises administering to the individual a therapeutically effective amount of an inhibitor (i.e., one or more inhibitors) of Structural Formula (I) or Structural Formula (II). For example, inflammatory diseases, including diseases which are associated with leukocyte infiltration of the gastrointestinal tract (including gut-associated endothelium), other mucosal tissues, or tissues expressing the molecule MAdCAM-1 (e.g., gut-associated tissues, such as venules of the lamina propria of the small

and large intestine; and mammary gland (e.g., lactating mammary gland)), can be treated according to the present method. Similarly, an individual suffering from a disease associated with leukocyte infiltration of tissues as a result of binding of leukocytes to cells (e.g., endothelial cells) expressing the molecule MAdCAM-1 can be treated according to the present invention.

Diseases which can be treated accordingly include inflammatory bowel disease (IBD), such as ulcerative colitis, Crohn's disease, ileitis, Celiac disease, nontropical Sprue, enteropathy associated with seronegative arthropathies, microscopic or collagenous colitis, eosinophilic gastroenteritis, or pouchitis resulting after proctocolectomy, and ileoanal anastomosis.

Pancreatitis and insulin-dependent diabetes mellitus are other diseases which can be treated using the present method. It has been reported that MAdCAM-1 is expressed by some vessels in the exocrine pancreas from NOD (nonobese diabetic) mice, as well as from BALB/c and SJL mice. Expression of MAdCAM-1 was reportedly induced on endothelium in inflamed islets of the pancreas of the NOD mouse, and MAdCAM-1 was the predominant addressin expressed by NOD islet endothelium at early stages of insulitis (Hanninen, A., *et al.*, *J. Clin. Invest.*, *92:* 2509-2515 (1993)). Further, accumulation of lymphocytes expressing $\alpha 4\beta 7$ within islets was observed, and MAdCAM-1 was implicated in the binding of lymphoma cells via $\alpha 4\beta 7$ to vessels from inflamed islets (Hanninen, A., *et al.*, *J. Clin. Invest.*, *92:* 2509-2515 (1993)).

Examples of inflammatory diseases associated with mucosal tissues which can be treated according to the present method include mastitis (mammary gland), cholecystitis, cholangitis or pericholangitis (bile duct and surrounding tissue of the liver), chronic bronchitis,

chronic sinusitis, asthma, and graft versus host disease (e.g., in the gastrointestinal tract). As seen in Crohn's disease, inflammation often extends beyond the mucosal surface, accordingly chronic inflammatory diseases of the lung which result in interstitial fibrosis, such as hypersensitivity pneumonitis, collagen diseases, sarcoidosis, and other idiopathic conditions can be amenable to treatment.

Some studies have suggested that the cell adhesion molecule, ICAM-1, can mediate leukocyte recruitment to inflammatory sites through adhesion to leukocyte surface ligands, i.e., Mac-1, LFA-1 or α4β1 (Springer, *Nature*, *346*:425-434 (1990)). In addition, vascular cell adhesion molecule-1 (VCAM-1), which recognizes the α4β1 integrin *(VLA-4)*, has been reported to play a role in *in vivo* leukocyte recruitment (Silber *et al.*, *J. Clin. Invest.* *93*:1554-1563 (1994)). It has been proposed that IBD can be treated by blocking the interaction of ICAM-1 with LFA-1 or Mac-1, or of VCAM-1 with α4β1 (e.g., WO 93/15764). However, these therapeutic targets are likely to be involved in inflammatory processes in multiple organs, and a functional blockade could cause systemic immune dysfunction. In contrast to VCAM-1 and ICAM-1, MAdCAM-1 is preferentially expressed in the gastrointestinal tract and mucosal tissues, binds the α4β7 integrin found on lymphocytes, and participates in the homing of these cells to mucosal sites, such as Peyer's patches in the intestinal wall (Hamann *et al.*, *J. Immunol.*, *152*:3282-3293 (1994)). As inhibitors of the binding of MAdCAM-1 to α4β7 integrin, the compounds of the present invention have the potential for fewer side effects due to e.g., effects on other tissue types where adhesion is mediated by other receptors, such as α4β1 integrin.

According to the method, an inhibitor can be administered to an individual (e.g., a human) alone or in conjunction with another agent, such as an additional pharmacologically active agent (e.g., sulfasalazine, an antiinflammatory compound, or a steroidal or other non-steroidal antiinflammatory compound). A compound can be administered before, along with or subsequent to administration of the additional agent, in amounts sufficient to reduce or prevent MAdCAM-mediated binding to a ligand for MAdCAM-1, such as human $\alpha4\beta7$.

An effective amount of an inhibitor can be administered by an appropriate route in a single dose or multiple doses. An effective amount is a therapeutically effective amount sufficient to achieve the desired therapeutic and/or prophylactic effect (such as an amount sufficient to reduce or prevent MAdCAM-mediated binding to a MAdCAM ligand, thereby inhibiting leukocyte adhesion and infiltration and associated cellular responses. Suitable dosages of inhibitors of Structural Formula (I) or (II) for use in therapy, diagnosis or prophylaxis, can be determined by methods known in the art and can be dependent, for example, upon the individual's age, sensitivity, tolerance and overall well-being. For example, dosages can be from about 0.1 mg/kg to about 50 mg/kg body weight per treatment.

A variety of routes of administration are possible including, but not necessarily limited to parenteral (e.g., intravenous, intraarterial, intramuscular, subcutaneous injection), oral (e.g., dietary), topical, inhalation (e.g., intrabronchial, intranasal or oral inhalation, intranasal drops), or rectal, depending on the disease or condition to be treated. Oral and parenteral administration are preferred modes of administration.

Formulation of an inhibitor to be administered will vary according to the route of administration selected (e.g., solution, emulsion, capsule). An appropriate

composition comprising the compound to be administered can be prepared in a physiologically acceptable vehicle or carrier. For solutions or emulsions, suitable carriers include, for example, aqueous or alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles can include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles can include various additives, preservatives, or fluid, nutrient or electrolyte replenishers (See, generally, Remington's Pharmaceutical Science, 16th Edition, Mack, Ed. (1980)). For inhalation, the compound can be solubilized and loaded into a suitable dispenser for administration (e.g., an atomizer, nebulizer or pressurized aerosol dispenser).

The present method can be used to assess the inhibitory effect of a compound of the present invention and of other potential antagonists useful in the method on the interaction of MAdCAM-1 with a ligand for MAdCAM-1 *in vitro* or *in vivo*. For example, compounds of the present invention were assayed for their ability to inhibit MAdCAM-1 binding to human $\alpha 4\beta 7$ integrin using an adhesion assay described in Example 2 and Example 3. Other suitable assays can be used to assess the ability of compounds to inhibit binding of MAdCAM-1 to a ligand for MAdCAM-1. For example, other fusion proteins (e.g., a chimeric protein or "immunoadhesin") can be constructed and used in an assay such as the assay described in Example 2 or other suitable assays. A fusion protein comprising human MAdCAM-1 or a portion thereof (e.g., the entire extracellular domain or the two N-terminal immunoglobulin domains) joined to an immunoglobulin heavy or light chain constant region can be produced and used in an assay similar to that described in Example 2, using a capture antibody suitable for the

immunoglobulin constant region selected. In a different assay, unlabeled cells bearing a MAdCAM-1 ligand can be contacted with such a fusion protein under conditions suitable for binding to the ligand in the presence or absence of compound, and the amount of bound chimeric protein determined (e.g., cells can be removed and the amount of chimeric protein bound determined by, flow cytometry or other suitable methods).

Compounds suitable for use in therapy can also be evaluated *in vivo*, using suitable animal models. Suitable animal models of inflammation have been described. For example, NOD mice provide an animal model of insulin-dependent diabetes mellitus. CD45 RB^{Hi}/SCID model provides a model in mice with similarity to both Crohn's disease and ulcerative colitis (Powrie, F. *et al.*, *Immunity, 1*: 553-562 (1994)). Captive cotton-top tamarins, a New World nonhman primate species, develop spontaneous, often chronic, colitis that clinically and histolgocially resembles ulcerative colitis in humans (Madara, J.L. *et al.*, *Gastroenterology, 88*: 13-19 (1985)). The tamarin model and other animal models of gastrointestinal inflammation using BALB/c mice (a (DSS)-induced inflammation model; DSS, dextran sodium sulfate) and common marmosets are described in Briskin *et al.*, U.S. Serial No. 08/523,004, filed September 1, 1995, the teachings of which are incorporated herein by reference in their entirety (see also Briskin *et al.*, WO 96/24673, published August 15, 1996). Knockout mice which develop intestinal lesions similar to those of human inflammatory bowel disease have also been described (Strober, W. and Ehrhardt, R.O., *Cell*, 75: 203-205 (1993)).

Preferably, selective inhibition of the interaction of MAdCAM-1 with a ligand thereof is achieved. Selective inhibition can be assessed, for example, by further

evaluating the effect of compounds on adhesion between one or more other receptor-ligand pairs. For example, adhesion assays which assess the interaction of a particular receptor and ligand pair, such as (a) VCAM-1 and $\alpha4\beta1$, (b) ICAM-1 and LFA-1, (c) fibronectin and $\alpha5\beta1$, and (d) fibronectin and $\alpha4\beta1$, can be conducted. Nonlimiting examples of suitable cell lines for such adhesion assays include (a) RAMOS cells, (b) JY cells, (c) K562 cells, and (d) RAMOS cells, respectively. In these assays, isolated and/or recombinant fibronectin (Sigma Chemical Co., St. Louis, MO), VCAM-1, ICAM-1, or fusion proteins comprising the ligand binding domain(s) of VCAM-1 or ICAM-1, can be used, for example.

The compounds of the present invention can also be used as immunogens to produce antibodies, including monoclonal and polyclonal antibodies, against MAdCAM-1 using methods known in the art or other suitable methods (see e.g., Kohler et al., *Nature*, *256*:495-497 (1975); Galfre, G., *et al.*, *Nature*, 299:550-552 (1977); Harlow et al., 1988, Antibodies: A Laboratory Manual, (Cold Spring Harbor, NY); or Current Protocols in Molecular Biology, Vol. 2 (Supplement 27, Summer '94), Ausubel et al., Eds. (John Wiley & Sons: New York, NY), Chapter 11 (1991)). Antibodies can be raised against an appropriate immunogen in a suitable mammal (e.g., a mouse, rat, rabbit or sheep). For example, a compound represented by Structural Formulas (I) and (II) or a variant thereof can be produced and used as an immunogen to raise antibodies in a suitable immunization protocol.

Antibody-producing cells (e.g., a lymphocyte) can be isolated from, for example, the lymph nodes or spleen of an immunized animal. The cells can then be fused to a suitable immortalized cell (e.g., a myeloma cell line), thereby forming a hybridoma. Fused cells can be isolated employing selective culturing techniques. Cells which

produce antibodies with the desired specificity can be selected by a suitable assay (e.g., ELISA).

Antibodies and monoclonal antibodies produced as described have a variety of uses. For example, those against or reactive with MAdCAM-1, and preferably which bind specifically to MAdCAM-1, can be used to identify and/or sort cells exhibiting MAdCAM-1 on the cell surface (e.g., in fluorescence activated cell sorting, histological analyses). Monoclonal antibodies specific for MAdCAM-1 can also be used to detect and/or quantitate MAdCAM-1 expressed on the surface of a cell or present in a sample (e.g., in an ELISA). Antibodies reactive with the immunogen are also useful. For example, they can be used to detect and/or quantitate immunogen in a sample, or to purify immunogen (e.g., by immunoaffinity purification).

This invention further relates to the diagnostic use or research use of antagonists of MAdCAM in the detection and/or quantitation of $\alpha4\beta7$ integrin present on leukocytes using a suitable label or indicator. For example, hydrocarbon fluorescence indicators such as a pyrene moiety, or radioisotope labels such as ^{125}I can be attached to the aryl ring of the N-terminus functional group of the inhibitor or antagonist peptide of this invention. The fluorescence properties of pyrene ring derivatives have been reported (I. A. Prokhorenko, *et al.*, *Bioorg. Med. Chem. Lett.*, 5:2081 (1995)). The use of labeled peptides for identifying cells having a membrane-bound protein is described in Riper *et al.*, *J. Exp. Med.* *177*:851 (1993).

This diagnostic tool would be valuable in the identification of $\alpha4\beta7$-positive leukocytes from subjects with suspected inflammatory bowel diseases and the like. Antagonist molecules of this invention possessing this indicator grouping are described as examples of the invention with adhesion antagonist properties.

EXEMPLIFICATION

The present invention will now be illustrated by the following Examples, which are not intended to be limiting in any way.

Example 1: Synthesis

The novel compounds of this invention can be synthesized according to the general procedures of synthesis, A-D, utilizing solid-phase peptide synthesis methodology described herein, which are known to person skilled in the art, or other suitable techniques. See e.g., Merrifield, R.B., *Science, 232*: 341 (1986); Carpino, L.A., Han, G.Y., *J. Org. Chem., 37*: 3404 (1972); Gauspohl, H., *et al., Synthesis, 5*: 315 (1992)).

For multiple peptide synthesis the Fmoc/t-Bu protocol was used. *In situ* activation of the amino acid derivative was performed by benzotriazolyl-N-oxytripyrrolidinophosphoniumhexaflurophosphate (PYBOP) or 2(1-benzotriazolyl-1-yl)-1,1,3,3-tetramethyluronium (HBTU) and N-methylmorpholine (NMM) in dimethylformamide (DMF) as a solvent. In order to improve the coupling efficiency and quality of the final peptides, each coupling reaction was carried out twice. As a solid support Rink Amide Am Resin (4-[2'-4-dimethoxyphenyl-Fmoc-aminomethyl]-phenoxyacetamido-norleucylaminoethyl resin) was used due to its high loading and excellent swelling properties. Fmoc group was removed by 10% piperidine and 2% DBU in DMF. The peptides were cleaved from the resin and the side chain protecting groups were simultaneously removed by a trifluoroacetic acid (TFA) cocktail. All peptides reported in Table 2 below are carboxy-terminus blocked as the carboxamide. More details of the peptide synthesis protocol are given below.

HPLC and mass spectral analysis

All of the crude peptides were analyzed by reversed phase HPLC using DELTAPAK C18, 5μm column, eluted with a linear gradient of 0.1% TFA in CH_3CN/water (100% CH_3CN/0% water) to 0.1% TFA in CH_3CN/water (0% CH_3CN/100% water) over a 30 minute period with flow rate of 1 ml/minute. The purity of the samples were determined and were essentially found to contain one component. This was confirmed by matrix-assisted laser desorption ionization time of flight mass spectral analysis (MALDI-TOF, Kratos, Inc.) internally referenced to leucine-enkephalin and sinapinic acid. Generally, the peptides gave the mass within 1%, of MH+ or M+Na+ or within experimental error of the calculated value.

General Procedure A. Peptide Synthesis

These peptides were synthesized via Fmoc/t-Butyl chemistry on a Gilson 421 automated multiple peptide synthesizer starting with Fmoc-Am-PS (100 mg, 0.5 mmol/g) resin. Acylations were carried out twice with the Fmoc-amino acid (5 equivalents), HBTU (5 equivalents)/NMM (10 equivalents) using 20-45 minute coupling time. Fmoc deprotection was carried out with 20% piperidine in DMF for 24 minutes. Treatment with Reagent-R (TFA-EDT-thioanisole-anisole, 90:5:3:2) for 2 hours was used to deblock and remove the peptides from the resin. The peptides were then precipitated from ether and lyophilized from acetic acid.

General Procedure B. Peptide Synthesis

Peptides were synthesized via Fmoc/t-Butyl chemistry on a Gilson 421 automated multiple peptide synthesizer starting with Fmoc-Am-PS (50 mg, 0.5 mmol/g) resin. Acylations were carried out twice with the Fmoc-amino acid (10 equivalents), HBTU (10 equivalents)/NMM (20 equivalents) or PYBOP (10 equivalents)/NMM (20 equivalents) using a 20-45 minute coupling time. Fmoc deprotection was

carried out with 2% 1,8-diazobicyclo[5,4.0]undec-7-ene (DBU) and 10% piperidine in DMF for 24 minutes. Treatment with Reagent-R (TFA-EDT-thioanisole-anisole, 90:5:3:2) for 2 hours was used to deblock and remove the peptides from the resin. The peptides were then precipitated from ether and lyophilized from acetic acid.

General Procedure C. Synthesis of N-Acylated Peptides

The peptides were synthesized via Fmoc/t-Butyl chemistry on a Gilson 421 automated multiple peptide synthesizer starting with Fmoc-Am-PS (50 mg, 0.5 mmol/g) resin. Acylations were carried out twice with the Fmoc-amino acid (10 equivalents), HBTU (10 equivalents)/NMM or PYBOP (10 equivalents)/NMM (20 equivalents) using 20-45 minute coupling time. For the final acylation the Fmoc-amino acid was substituted with an appropriate organic acid. Fmoc deprotection was carried out with 2% DBU and 10% piperidine in DMF for 24 minutes. Treatment with Reagent-R (TFA-EDT-thioanisole-anisole, 90:5:3:2) for 2 hours was used to deblock and remove the peptides from the resin. The peptides were then precipitated from ether and lyophilized from acetic acid.

General Procedure D. Synthesis Peptides Cyclized Through A Disulfide Bond

The linear acyclic peptides were synthesized as described above in the General Procedure B. The acetamidomethyl (Acm) protecting group from the cysteine side chain was simultaneously removed and peptides were cyclized by iodine treatment. A solution of 25 mg of iodine in 5 mL of 80% aqueous acetic acid was added to 5 mg of peptide. The mixture was shaken at room temperature for 2 hours, diluted with water (25 ml), extracted with chloroform (3 X 25 ml) and finally lyophilized to give the cyclized peptide.

Example 2: Biological Activity

The compounds of the present invention were evaluated for their biological properties. Table 2 lists a number of compounds of the present invention, their physical characterization, and their ability to inhibit the adhesion of α4β7-bearing cells (HUT 78 cells) to MAdCAM-1 (described as percent inhibition at the concentration noted or by the corresponding IC_{50} value (μm) determined using the adhesion assay described below. IC_{50} corresponds to the concentration of compound which inhibits 50% of the total number of cells adhering to MAdCAM-1 in a control conducted in the absence of inhibitor.

Overview

A soluble fusion protein comprising murine MAdCAM-1 was produced in a baculovirus expression system and used in the adhesion assay. A fusion gene in which sequences encoding the signal peptide and extracellular domain of murine MAdCAM-1 were fused to sequences encoding a constant region of the murine kappa light chain (mC_κ) was constructed, and cloned into a baculovirus shuttle vector. Fusion protein produced from the resulting construct contained the integrin binding sequences of murine MAdCAM-1 at the amino-terminus, and the mCκ sequence at the carboxy-terminus. Recombinant baculovirus encoding the fusion protein was harvested from infected Sf9 insect cells. Fusion protein was detected in supernatants of infected Sf9 cells by ELISA assay, using a horseradish peroxidase-linked polyclonal anti-mCκ antibody and chromogenic substrate according to standard protocols. Recombinant protein was also verified by immunoprecipitation with anti-murine MAdCAM-1 monoclonal antibody (MAb MECA-367).

For adhesion assays, a dose response curve obtained using increasing amounts of rat anti-mCκ MAb indicated the amount of rat anti-mCκ MAb to be used as capture antibody

in the assay. Subsequently, an IC50 for Ac-LDTSL-NH2 was determined to be 278 μM.

Human T-cell lymphoma cells (HUT 78 cells) activated with Mn^{+2} were used for the adhesion assay in a 96-well format. HUT 78 cells were labelled by preincubation with BCECF-AM stain (Molecular Probes). Assays were conducted in a final volume of 200 μl. The adhesion of HUT 78 cells to MAdCAM-1/mCκ fusion protein bound to wells via rat anti-mCκ capture antibody was assessed in the presence or absence of each compound. Adhesion of HUT 78 cells was monitored using a fluorescent plate reader at a setting gain of 10 at 485/535 nM. Percent inhibition and IC_{50} values were determined.

Production of MAdCAM-1/mCκ Fusion Protein

Antibodies, Cells and Viruses

Affinity-purified polyclonal goat-anti-mouse-C-kappa (mC_κ) antibodies (#M33100), horseradish peroxidase-linked goat anti-mC_κ antibodies (#M33107), and alkaline phosphatase-linked swine anti-goat H & L chains (#G50008) were purchased from Caltag. Sepharose-linked rat anti-mC_κ affinity matrix was obtained from Zymed.

Affinity purified rat-anti-mC_κ monoclonal antibodies were prepared by Dr. Hans Peter Kocher (BTC) from the rat hybridoma cell line 187.1 (American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852, Accession No. ATCC HB 58), and were linked to cyanogen bromide-activated Sepharose 4B for affinity purification of proteins.

ACMNPV DNA and cationic liposomes (Invitrogen transfection kit #B825-03) or lipofectin (GIBCO/BRL #82925A) were used to transfect Sf9 insect cells (American Type Culture Collection, Accession No. ATCC CRL 1711; gift from Dr. Max Summers) in HyQ serum-free medium (Hyclone, #B-5502-L-P). BaculoGold (Pharmingen) was also used in

some transfections. Cells were maintained in TNM-FH medium (GIBCO BRL) supplemented with 10% fetal bovine serum and 0.1% F-68 pluronic acid (GIBCO/BRL, #670-404AG).

Construction of pVL941/mC$_\kappa$

Based on the published sequence for the mouse kappa constant region (mC$_\kappa$; Hieter, P.A., *et al.*, *Cell 22*: 197-207 (1980)), two oligonucleotides having the following sequences (SEQ ID NO: 5 and SEQ ID NO: 6, respectively) were designed and synthesized:

5' primer:

5'-GGATCC GCT GAT GCT GCA CCA ACT GTA TTC-3'

3' primer:

5'-CCT TTG TCC TAA CAC TCA TTC CTG TT-3'

The mC$_\kappa$ coding sequence was amplified by polymerase chain reaction (PCR) from the plasmid pVL91A3 containing the full-length 91A3 mouse kappa light chain (Meek, K., *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, *84*: 6244-6248 (1987)). An in-frame BamHI restriction site (underlined in the sequence above) was incorporated at the 5'-end of the coding sequence to facilitate fusion with MAdCAM-1 sequences.

Vector pVL941 (Invitrogen) was linearized by digestion at the unique BamHI site in the polyhedrin gene, and the ends were made blunt with the Klenow fragment of DNA polymerase. The amplified DNA fragment (312 base pairs) containing the 91A3 mouse kappa light chain was then ligated to pVL941 to yield pVL941/mC$_\kappa$. Clones containing the mC$_\kappa$ sequence in the same 5' to 3' orientation as the polyhedrin promoter were selected.

Construction of Baculovirus Shuttle Vector

Based on the published nucleotide sequence of murine MAdCAM-1 (Briskin, M.J. *et al.*, *Nature, 363:* 461-464 (1993)), oligonucleotide primers having the following sequences were designed and synthesized:

5' primer:

5'-GAT CAG GGA TCC **ATG** GAA TCC ATC CTG GCC CTC CTG-3'

3' primer:

5'-TCG ATC GGA TCC GGT GGA GGA GGA ATT CGG GGT CA-3'

The ATG start codon of MAdCAM-1 is indicated in bold. The 5' and 3' primers each incorporated a BamHI restriction site for cloning into the expression vector (underlined above). Murine MAdCAM-1 sequences were amplified by PCR using these 5'- and 3'-primers. The product was digested with BamHI and inserted into vector pVL941/mCκ, which had been digested with BamHI. DNA fragments cloned into the BamHI site were screened for the correct orientation with respect to the mCκ sequence. Fusion proteins produced from the resulting construct contained mouse MAdCAM-1 sequences at the N-terminus, and the mC_κ sequence at the carboxy-terminus (Figure 3). A two-amino acid glycine-serine spacer encoded by the six nucleotides corresponding to the BamHI restriction site forms the junction between these sequences.

Transfection of Sf9 Insect Cells

Co-transfection (lipofection) of Sf9 insect cells with a mixture of 1 μg of ACMNPV DNA (Invitrogen), 3-5 μg of shuttle vector DNA (pVL941/MAdCAM-1/mCκ purified by magic-mini prep, Promega) and cationic liposomes (30 μl) was performed according to the manufacturers' instructions, with slight modifications. 2 x 10^6 μ cells in TNM-FH

medium (10% FBS) were plated out in 60 mm dishes and allowed to attach over a 2-4 hour period prior to transfection. The medium was removed and the adherent cells were washed twice with serum-free medium (HyQ). Three ml of HyQ containing the DNA/liposome mixture were applied dropwise over the cells; the cells were incubated overnight. The medium was then removed by aspiration and 5 ml TNF-FN medium containing 10% FBS was added to each dish. After 48 hours, one ml was removed for plaque purification of recombinant virus, as previously described (O'Reilly, D.R., *et al.*, (Eds.), *Baculovirus Expression Vectors* (W.H. Freeman and Co.: New York), pp. 124-128 (1992)).

<u>ELISA Assay of Transfected Sf9 Cells</u>

For each transfection, triplicate wells were coated with polyclonal goat anti-mC_κ antibodies (2 μg/ml in carbonate/bicarbonate buffer), and incubated overnight. The plates were washed three times with phosphate buffered saline (PBS) containing 0.5% Triton X-100, and 0.5 M NaCl and then washed twice with PBS. This washing protocol was used between all steps. The wells were then blocked with 2% BSA in TBS for one hour. Supernatant (100 μl) taken from cells four to five days post-infection was applied to each well and incubated at 37°C for 2 hours. The presence of mC_κ fusion protein was detected by the addition of horseradish peroxidase-linked polyclonal anti-mC_κ antibody and chromogenic substrate, according to standard protocols.

<u>Imunodetection of mC_κ fusion proteins</u>

Recombinant virus containing the MAdCAM-1/mCκ fusion gene was isolated by plaque purification, and amplified by infecting Sf9 insect cells (2 x 10^6 cells/60 mm petri dish) in 5 ml TNM-FH medium. From this 5 ml stock, an aliquot (100 μl) was taken to infect 2 X 10^6 Sf9 cells as before. Supernatants (10 μl) taken 24, 48, 72 and 96 hours post-

infection were assayed for the presence, accumulation and stability of mC_κ fusion protein by Western blot analysis. Samples were applied to 10% SDS/PAGE gels. Proteins were electrophoretically transferred to nitrocellulose by standard techniques. The fusion protein was detected by addition of polyclonal goat anti-mC_κ antibodies (2 μg) in 5 ml BLOTTO (5%) followed by treatment with alkaline phosphatase-linked swine anti-goat H & L chains and chromogenic substrate (BIORAD, Catalog No. 170-6432).

<u>Purification of mC_κ fusion protein by affinity chromatography</u>

Production of fusion proteins was carried out in stirred microcarrier flasks. Sf9 insect cells (2×10^6 cells/ml) were infected with virus particles containing the MAdCAM-1/mCκ fusion gene at a multiplicity of infection of 0.001. Upon complete lysis of the cells (approximately eight days later), the spent medium was clarified by low speed centrifugation. The supernatant was applied to a rat monoclonal anti-mCκ-coupled Sepharose column (purchased from Zymed or prepared using rat monoclonal 187.1) equilibrated in TBS pH 7.0. The column was washed with TBS pH 7.0, and the bound mC_κ fusion protein eluted in glycine buffer pH 2.2 and neutralized by the addition of 2M Tris pH 8.0. Yields of 2 mg of mC_κ fusion protein per liter of cells were obtained.

<u>Results</u>

MAdCAM-1/mC_κ fusion protein was expressed under the transcriptional regulation of the viral polyhedrin promoter. To transfer the chimeric gene into the baculovirus genome, pVL941/MAdCAM-1/mC_κ plasmid was co-transfected with *A. californica* nuclear polyhedrosis virus DNA into Sf9 insect cells. Several recombinant plaques

were isolated and one was chosen for more detailed characterization.

ELISA assays on Transfected Cell Supernatants

To determine if an ELISA assay could be used to detect the appearance of fusion protein in the supernatant of transfected cells, aliquots (500 μl) were taken from the supernatant of transfected cells at 3, 4 and 5 days post-transfection, and ELISA assays were performed as described above. The results indicated that the sensitivity of the ELISA assay was sufficient to detect the mC_κ fusion protein. The assay can be used to provide an indication that the desired gene is expressed in the insect cells. Moreover, a positive signal in the ELISA assay correlated well with the isolation of recombinant viral particles in subsequent plaque assays.

Cellular Adhesion Assay

The following buffers and reagents were used in the initial titration of capture antibody and in the adhesion inhibition assays:

Table 1

CARBONATE BUFFER:

17.2 g $NaHCO_3$	Sigma	#S-8875	
8.6 g Na_2CO_3	Sigma	#S-6139	
Bring volume to 1L with H20			

1% BSA/PBS

BSA	5 g	Sigma	#A-6793
PBS	500 ml	GIBCO	#14040-026
Sterile filter			

ASSAY BUFFER (HESS / 2%FCS / 25mM HEES / Pinstripe., pH 7.2):

HESS	500 ml	Gibco	#14025-02
FCS	10 ml	Gibco	#16000-044
HEES (1M)	12.5 ml	Gibco	#15630-015
Pen.Strep. (100X)	5 ml	Gibco	#15070-022
Sterile filter			

2X ASSAY BUFFER (2X HBSS / 4%FCS / 50mM HEPES / Pen.Strep, pH 7.2):

10X HBSS	100 ml	Gibco	#14065-023
FCS	20 ml	Gibco	#16000-044
HEPES(1M)	25 ml	Gibco	#15630-015
$NaHCO_3$(7.5%)	4.7 ml	Gibco	#25080
Pen.Strep.	10 ml	Gibco	#15070-022
Check pH			
Bring volume up to 500 ml with H2O			
Sterile filter			
BCECF-AM		Molecular Probes	#B-1170
DMSO		Sigma	#D-8779

Maintenance and Labelling of Cells

HUT 78 cells (a human T cell lymphoma line; American Type Culture Collection, Accession No. ATCC TIB 161) were maintained in culture for up to one month in RPMI 1640 supplemented with 10% FCS, 20 mM HEPES, and Pen-Strep (1:100). Just prior to use in adhesion assays, cells were labeled with BCECF-AM as follows: 1 X 10^7 cells were pelleted at 1000 rpm for 10 minutes and resuspended in 25 mL cold PBS (Phosphate Buffered Saline). The cells were pelleted again, resuspended in cold PBS, and pelleted again. The cell pellet was resuspended in 25 ml PBS and labeled with 50 µl BCECF-AM (1 µg/µL in DMSO) for 30 minutes at 37°C. (BCECF-AM; 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyflourescein, acetoxymethyl ester). The labeled cells were pelleted at 700 rpm for 10 minutes, resuspended in 25 ml cold Assay Buffer and pelleted. Finally, labeled cells were counted and resuspended in Assay Buffer (ambient temperature) at a concentration of 2.5 X 10^6/ml.

Titration of Capture Antibody

A rat anti-mCκ antibody (affinity purified rat-anti-m$C_κ$ monoclonal antibodies from rat hybridoma cell line 187.1; ATCC Accession No. HB58) was selected for use as capture antibody in the adhesion assay. A titration experiment was conducted in order to determine the optimal capture antibody concentration. Assay Buffer for the titration was 2X Assay Buffer.

ELISA plates were coated with various concentrations capture antibody by adding 10 µg/ml, 5 µg/ml, 2.5 µg/ml, or 1.25 µg/ml of antibody in a 50 µl volume to each well and incubating at 4°C overnight. The next day, plates were blocked with 100 µl 1% BSA/PBS at 37°C for 1 hour. After one hour, the BSA/PBS solution was removed, and 50 µl

MAdCAM-mCκ fusion protein (neat supernatant) was added to each well and incubated for 1 hour at 37°C.

Anti-murine MAdCAM-1 antibody MECA-367 (American Type Culture Collection, Accession No. ATCC HB 9478) was used as a blocking antibody. For the titration, either 20 μl of Assay Buffer (i.e., 2X Assay Buffer), 20 μl of neat supernatant containing anti-MAdCAM-1 MECA-367 antibody, or 20 μl of neat supernatant containing MECA-367 diluted 1:2, 1:4, 1:8 or 1:16 in Assay Buffer, was added to each well. The undiluted supernatant contained approximately 3 μg/ml of antibody.

Frozen BCECF-labeled HUT 78 cells were thawed and resuspended in Assay Buffer at 2.5 X 10^6 cells/ml. 50 μl of cells, 50 μl of Assay Buffer (without Pen/Strep), 30 μl of water, 50 μl of 8 mM $MnCl_2$, and 20 μl of either MECA-367 antibody (neat or diluted) or 20 μl of Assay Buffer alone, were then added to each well, and the plates were incubated on a rotator for 30 minutes at room temperature.

After incubation, a baseline measurement for each well of total fluorescence was taken using a Fluorescence Concentration Analyzer (IDEXX) at 485/535 nM. Then plates were washed twice with a solution of 50 mM Tris / 2 mM $MnCl_2$ using an EL 404 Microplate Autowasher (BIO-TEK Instruments), and fluorescence (due to adherent cells) was determined again using a Fluorescence Concentration Analyzer (IDEXX) at 485/535 nM. For each well, the final reading was divided by the baseline reading. The values from duplicate wells were averaged, and plotted (Figure 1). Accordingly, 50 μl of a 5 μg/mL solution of rat anti-mCκ was used to coat each well for subsequent adhesion assays.

Inhibition Assays and IC_{50} Determination

The assay was performed in a 96-well format. Plates were coated overnight at 4°C with 50 μl/well of a 5 μg/mL solution of rat anti-mCκ in carbonate buffer. The plates were blocked for 1 hour at 37°C with 100 μL/well of 1% BSA in PBS. The BSA/PBS solution was removed, and 50 μL of MAdCAM-mCκ supernatant was added neat to each well. The assay was conducted under conditions in which capture antibody was the limiting reagent.

Cell adhesion was measured in the presence or absence of compounds. The compounds were resuspended in 100% DMSO, subsequently diluted 1:10 in water, and finally diluted again 1:10 in the assay: 50 μL of 2X Assay Buffer, 30 μL water, 20 μL of compound were added to each well. 50 μL of a cell suspension containing BCECF-AM labeled HUT 78 cells (resuspended in assay buffer at a concentration of 2.5×10^6/ml; see above) were added to each well, followed by 50 μL of a solution of 8 mM $MnCl_2$ in assay buffer. Cell adhesion occurred in 30 minutes at ambient temperature, after which plates were washed with 50 mM Tris/2 mM $MnCl_2$, pH 7.2 using an EL 404 Microplate Autowasher (BIO-TEK Instruments) using the following wash parameters: wash volume = 500 μL; two wash cycles; wash depth = 80; aspirating after each wash. Plates were read using a Fluorescence Concentration Analyzer (IDEXX) at 485/535 nM.

IC_{50} Determinations

In order to determine an IC_{50} (the concentration of compound which inhibits 50% of the total number of cells adhering to MAdCAM-1 relative to a control conducted in the absence of inhibitor), compounds were tested for inhibition of HUT 78 cell adhesion as described above at various concentrations ranging between ~62.5 to 500 μM. Additional determinations were conducted as needed, so that each IC_{50} was determined over a range which encompassed the IC_{50}.

Over this range, four different concentrations were tested in duplicate. For example, compound 793-1a was tested at four different concentrations (50 μM, 25 μM, 12.5 μM and 6.25 μM) in duplicate wells.

For each compound, a ratio was determined as follows: An average of the readings from duplicate wells was divided by an average of eight control wells (adhesion in the absence of any compound). Percent inhibition of adhesion was calculated as 100 x (1 - the ratio). In addition, the concentration (μM) of compound used was plotted against the resulting ratios. The resulting plot for compound 793-1a is shown in Figure 2.

The IC_{50} was then determined using Kaleidograph software (Abelbeck Software). The complete procedure was repeated again using duplicate wells for each point. An average of two IC_{50} determinations was obtained, and the resulting values are presented in Table 2 with percent adhesion inhibition for a variety of compounds of the present invention. For example, the IC_{50} for compound 793-1a was determined to be 14 μM. Cysteines in cyclic peptides which are linked by a disulfide bond are indicated by an "*". Abbreviations used are defined in Table 3 below.

In Table 2, Example No. 1 is represented by SEQ ID NO: 7; Example No. 2 is represented by SEQ ID NO: 8; Example No. 3 is represented by SEQ ID NO: 9; Example No. 4 is represented by SEQ ID NO: 10; Example No. 5 is represented by SEQ ID NO: 11; Example No. 6 is represented by SEQ ID NO: 12; Example No. 7 is represented by SEQ ID NO: 13; Example No. 8 is represented by SEQ ID NO: 14; Example No. 9 is represented by SEQ ID NO: 15; Example No. 10 is represented by SEQ ID NO: 7; Example No. 11 is represented by SEQ ID NO: 16; Example No. 12 is represented by SEQ ID NO: 17; Example No. 13 is represented by SEQ ID NO: 18; Example No. 14 is represented by SEQ ID NO: 9; Example No.

15 is represented by SEQ ID NO: 13; Example No. 16 is represented by SEQ ID NO: 19; Example No. 17 is represented by SEQ ID NO: 20; Example No. 18 is represented by SEQ ID NO: 21; Example No. 19 is represented by SEQ ID NO: 22; Example No. 20 is represented by SEQ ID NO: 23; Example No. 21 is represented by SEQ ID NO: 24; Example No. 22 is represented by SEQ ID NO: 25; Example No. 23 is represented by SEQ ID NO: 26; Example No. 24 is represented by SEQ ID NO: 7; Example No. 25 is represented by SEQ ID NO: 27; Example No. 26 is represented by SEQ ID NO: 28; Example No. 27 is represented by SEQ ID NO: 29; Example No. 28 is represented by SEQ ID NO: 30; Example No. 29 is represented by SEQ ID NO: 31; Example No. 30 is represented by SEQ ID NO: 32; Example No. 31 is represented by SEQ ID NO: 33; Example No. 32 is represented by SEQ ID NO: 34; Example No. 33 is represented by SEQ ID NO: 35; Example No. 34 is represented by SEQ ID NO: 36; Example No. 35 is represented by SEQ ID NO: 37; Example No. 36 is represented by SEQ ID NO: 38; Example No. 37 is represented by SEQ ID NO: 39; Example No. 38 is represented by SEQ ID NO: 40; Example No. 39 isple No. 40 is represented by SEQ ID NO: 42; Example No. 41 is represented by SEQ ID NO: 43; Example No. 42 is represented by SEQ ID NO: 44; Example No. 43 is represented by SEQ ID NO: 45; Example No. 44 is represented by SEQ ID NO: 46; Example No. 45 is represented by SEQ ID NO: 47; Example No. 46 is represented by SEQ ID NO: 48; Example No. 47 is represented by SEQ ID NO: 49; Example No. 48 is represented by SEQ ID NO: 50; Example No. 49 is represented by SEQ ID NO: 51; Example No. 50 is represented by SEQ ID NO: 52; Example No. 51 is represented by SEQ ID NO: 53; Example No. 54 is represented by SEQ ID NO: 54; Example No. 55 is represented by SEQ ID NO: 55; Example No. 56 is represented by SEQ ID NO: 56; Example No. 57 is represented by SEQ ID NO: 57; Example No. 58 is represented by SEQ ID NO: 7; Example No. 59 is represented by SEQ ID NO: 58;

Example No. 60 is represented by SEQ ID NO: 59; Example No. 61 is represented by SEQ ID NO: 60; Example No. 62 is represented by SEQ ID NO: 61.

Table 2

Ex No	L#	Sequence	Method Prep	MH+ Cal. Found	% Purity HPLC	% Adhesion Inhibition 500 μM	IC50 (μM)	
1	00112-1A	Ac-Leu-Asp-Thr-Ser-Leu	B	591	616	75		278
2	00619-1A	Ac-Leu-Asp-Ala-Ser-Leu	B	557	563	78	47	1004
3	0092-1A	Ac-His-Trp-Arg-Gly-Leu-Asp-Thr	B	926	926	50	49	584
4	00167-1B	Ac-Cys*-Leu-Asp-Thr-Ser-Leu-Cys*	B,D	797	816	67	51(@ 250 μM)	102.4
5	00145-1B	Ac-Cys*-Gly-Leu-Asp-Thr-Ser-Leu-Gly-Cys*	B,D	909	925	80	45(@ 200 μM)	266
6	0093-1A	Ac-Trp-Arg-Gly-Leu-Asp-Thr-Ser	B	877	878	42	44	862
7	0096-1A	Ac-Trp-Arg-Gly-Leu-Asp-Thr	B	789	792	53	46	725
8	0094-1A	Ac-Arg-Gly-Leu-Asp-Thr-Ser-Leu	B	804	802	34,41	35	1575
9	0099-1A	Ac-Gly-Leu-Asp-Thr-Ser-Leu	B	648	647	67	58	339
10	0104-1A	Ac-Leu-Asp-Thr-Ser-Leu	B	591	616	47	50	530
11	0093-1A	Ac-Trp-Arg-Gly-Leu-Asp-Thr-Ser	B	877	878	42	44	862
12	0097-1A	Ac-Arg-Gly-Leu-Asp-Thr-Ser	B	691	691	32,34	31	1789

Table 2 (Cont'd)

13	0108-1A	Ac-Gly-Leu-Asp-Thr-Ser	B	535	554	73	43	745
14	0092-1A	Ac-His-Trp-Arg-Gly-Leu-Asp-Thr	B	926	926	50	49	584
15	0096-1A	Ac-Trp-Arg-Gly-Leu-Asp-Thr	B	789	792	53	46	725
16	0105-1A	Ac-Arg-Gly-Leu-Asp-Thr	B	603	607	45	48	542
17	00379-1A	Ac-MeLeu-Leu-Asp-Thr-Ser-Leu	B	965	966	50	28	1316
18	00380-1A	Phx-Leu-Asp-Thr-Ser-Leu	B,C	722	722		32	818
19	00381-1A	Impa-Leu-Asp-Thr-Ser-Leu	B,C	736	737		44	1140
20	00382-1A	Bipa-Leu-Asp-Thr-Ser-Leu	B,C	742	765	80	54	485
21	00383-1A	Npa-Leu-Asp-Thr-Ser-Leu	B,C	716	713	38,48	39	548
22	00384-1A	Hbz-Leu-Asp-Thr-Ser-Leu	B,C	750	770	77	51	401
23	00386-1A	Pba-Leu-Asp-Thr-Ser-Leu	B,C	818	817	94	88(@ 50 μM)	2.8
24	00389-1A	Ac-Leu-Asp-Thr-Ser-Leu	B,C	710	711	61	29	205
25	00391-1A	Tpc-Leu-Asp-Thr-Ser-Leu	B,C	672	670	88	39	121
26	00392-1A	Pha-Leu-Asp-Thr-Ser-Leu	B,C	666	693	86	38	2128
27	00393-1A	MeLeu-Leu-Asp-Thr-Ser-Leu	B,C	905	928	86	54	256
28	00710-1A	Tpa-Leu-Asp-Thr-Ser-Leu	B,C	813	837	74		0.3

Table 2 (Cont'd)

29	00711-1A	Paa-Leu-Asp-Thr-Ser-Leu	B,C	785	790	81		0.5
30	00781-1A	Pba-Pro-Leu-Asp-Thr-Ser-Leu	B,C	910	912	77		47
31	00784-1A	Dpa-Leu-Asp-Thr-Ser-Leu	B,C	737	765	87		11
32	00785-1A	Dphb-Leu-Asp-Thr-Ser-Leu	B,C	799	807	87		15
33	00786-1A	Flc-Leu-Asp-Thr-Ser-Leu	B,C	735	739	71		17
34	00787-1A	Pca-Leu-Asp-Thr-Ser-Leu	B,C	771	799	80		2.1
35	00788-1A	Xnc-Leu-Asp-Thr-Ser-Leu	B,C	751	757	84		3
36	00789-1A	Pba-Leu-Asp-Thr-Ser-Leu	B,C	813	818	81		4
37	00793-1A	Tphp-Leu-Asp-Thr-Ser-Leu	B,C	823	831	87		14
38	00795-1A	2-Anc-Leu-Asp-Thr-Ser-Leu	B,C	747	756	63		5.4
39	00603-1A	Pba-Leu-Ala-Thr-Ser-Leu	B,C	771	775	84	100(@ 250 μM)	28
40	00605-1A	Ac-Leu-Phe-Thr-Ser-Leu	B	623	623	86	29	299.7
41	00606-1A	Ac-Leu-Glu-Thr-Ser-Leu	B	605	605	85	98	60.7
42	00610-1A	Pba-Leu-Tyr-Thr-Ser-Leu	B,C	862	867	95	100	30
43	00612-1A	Ac-Leu-Asp-Ser-Ser-Leu	B	578	580	86	38	611.4
44	00613-1A	Ac-Leu-Asp-Thr-Thr-Leu	B	604	609	71	98	100.7

Table 2 (Cont'd)

45	00614-1A	Ac-Leu-Asp-Thr-Ser-Phe	B	625	625	80	86	143.9
46	00615-1A	Ac-Lle-Asp-Thr-Ser-Leu	B	591	593	79	17	18983
47	00712-1A	Ac-Leu-Asp-Val-Ser-Leu	B	589	611	93		71
48	00716-1A	Ac-Leu-Asp-HyP-Ser-Leu	B	599	626	79		106
49	00718-1A	Ac-MeLeu-Asp-Thr-Ser-Leu	B	587	629	65		150
50	00799-1A	Cpc-Asp-Thr-Ser-Leu	B,C	526	532	73		8.9
51	00649-1A	Pba-Asp-Thr-Ser-Leu	B,C	702	704	85	100(@ 100 μM)	13.3
52	00800-1A	Pca-Asp-Thr	B,C	459	460	76		11
53	00650-1A	Pba-Asp-Thr	B,C	503	505	96	100(@ 100 μM)	13.5
54		NH2-Leu-Asp-Thr-Ser-Leu					22	
55		NH2-Trp-Arg-Gly-Leu-Asp-Thr-Ser-Leu-Gly-Ser					76	
56		NH2-His-Trp-Arg-Gly-Leu-Asp-Thr-Ser-Leu-Gly-Ser-Val					80	
57		NH2-Arg-Val-His-Trp-Arg-Gly-Leu-Asp-Thr-Ser-Leu-Gly-Ser-Val-Gln					90	
58		Ac-Leu-Asp-Thr-Ser-Leu					71	
59		Ac-Arg-Gly-Leu-Asp-Thr-Ser-Leu-Gly					75	

Table 2 (Cont'd)

60	Ac-Trp-Arg-Gly-Leu-Asp-Thr-Ser-Leu-Gly-Ser	71
61	Ac-His-Trp-Arg-Gly-Leu-Asp-Thr-Ser-Leu-Gly-Ser-Val	71
62	Ac-Arg-Val-His-Trp-Arg-Gly-Leu-Asp-Thr-Ser-Leu-Gly-Ser-Val-Gln	88

Table 3

Ac = acetyl
Anc = 2 Anthracenecarbonyl
Bipa = 4-biphenylacetyl
Bpc = benzofuranecarbonyl
Chc = cyclohexanecarbonyl
Cpa = 1-cyclopentylacetyl
Cpc = cyclopentanecarbonyl
DBU = diazobicyclo [5,40] undec-7-ene
DMF = N,N-dimethylformanide
Dpa = diphenylacety
Dphb = 3,5-diphenylbenzoyl
EDT = 1,2-ethanedithiol
Fc = furanecarbonyl
HBTU = 2(1-benzotriazolyl-1-yl)-1,1,3,3-tetramethyluronium
Hbz = 4-heptylbenzoyl
Hyp = 4-hydroxyproline
Idc = Indolecarbonyl
Impa = 4-isobutyl-a-methylphenylacetyl
Indc = Indanecarbonyl
NMM = N-methylmorpholine
Npa = a-naphthylacetyl
Paa = 1-pyreneacetyl
Pba = 1-pyrenebutyryl
Pca = 1-pyrenecarbonyl
Pha = phenylacetyl
Phx = 6-phenylhexanoyl
PYBOP = benzotriazolyl-N-oxytripyrrolidinophosphoniumhexafluorophosphate
Rink Amide Am Resin = 4-[2',4'-dimethoxyphenyl-Fmoc-aminomethyl]-phenoxylacetamido-norlecucylaminomethyl resin
Tcc = trans-cinnamoyl
TFA = trifluoroacetic acid
Thc = Thienylcarbonyl

Tpa = triphenylacetyl

Tpc = tetramethylcyclopropylcarbonyl

Tpip = triphenylpropionyl

Xnc = xanthenecarbonyl

Example 3: Human MAdCAM Adhesion Assay

Design and functional analysis of a human MAdCAM-1-IgG (huMAdCAM-1-Ig) chimera

Construction of huMAdCAM-IgG Chimera

Human MAdCAM-1 clone 4 cDNA (Figure 4, SEQ ID NO: 67), present in vector pCDNA3 (Invitrogen, San Diego, CA.), was used as a template for PCR amplification of extracellular regions of human MAdCAM-1 to be fused with the constant region of a human IgG1. The human MAdCAM-1 clone 4 cDNA/pcDNA3 construct is also referred to as pcD3huMAd4 or pCDhuMAd4 (see Briskin *et al.*, WO 96/24673, published August 15, 1996, Example 1; see also Shyjan, A.M. *et al.*, *J. Immunol.*, *156:* 2851-2857 (1996), the teachings of which are each incorporated herein by reference). In particular, primer HUMADIG4/2 (SEQ ID NO: 83), which contains the 5' end of human MAdCAM-1 coding sequence (ATG codon, bold), was synthesized:

HUMADIG4/2 (SEQ ID NO: 83),

HindIII
5'-GGAAGCTTCCACC**ATG**GATTTCGGACTGGCCC-3'

This 5' primer was used in conjunction with a 3' primer to amplify a region encoding the entire extracellular domain of human MAdCAM-1 (clone 4). The 3' primer, designated HUMADIG3 (SEQ ID NO: 84), which contains a portion complementary to coding strand nucleotides 992-1010 of SEQ ID NO: 67, had the following sequence:

HUMADIG3 (SEQ ID NO: 84)

SpeI
5'-GGACTAGTGGTTTGGACGAGCCTGTTG-3'

The primers were designed with a 5' HindIII site or 3' SpeI sites as indicated. These primers were used to PCR amplify a MAdCAM fragment, using a PCR optimizer kit from Invitrogen (San Diego, CA). The PCR product was digested with the enzymes HindIII and SpeI to generate ends for cloning, and was purified by gel electrophoresis using the Glassmax DNA isolation system (Gibco, Bethesda, MD).

A ~1 kb fragment encompassing the CH1, H (hinge), CH2 and CH3 constant regions was excised by digestion with SpeI and EcoRI from a construct encoding a human immunoglobulin γ1 heavy chain, having a constant region described by Reichmann, L. *et al., Nature, 322*: 323-327 (1988) and Takahashi, N. *et al., Cell, 29:* 671-679 (1982)), which further contained two mutations in the Fc region. The mutations in the Fc region of this Ig heavy chain construct (Leu^{235} → Ala^{235} and Gly^{237} → Ala^{237}) were designed to reduce binding to human Fcγ receptors, and were produced by oligonucleotide-directed mutagenesis. The antibody encoded by this construct was used as an isotype matched irrelevant control hereinbelow. The 1 kb SpeI-EcoRI fragment encoding the Fc-mutated IgG1 constant region was isolated by gel electrophoresis using the Glassmax DNA isolation system (Gibco, Bethesda MD). This constant region fragment and the HindIII-SpeI fragment containing the entire extracellular domain were ligated in a three-way ligation to vector pEE12 (Stephens, P.L. and M.L. Cockett, *Nucl. Acids Res., 17*: 7110 (1989) and Bebbington, C.R. and C.C.G. Hentschel, 1987, *The use of vectors based on gene amplification for the expression of cloned genes in mammalian cells*, (Academic Press, N.Y.)), which had been

digested with HindIII and EcoRI. Transformants of the bacterial strain DH10B were obtained. Colonies were grown and mini plasmid preps were analyzed by restriction mapping. A construct designated HuMAdIg21 (from clone 21), which encodes a fusion protein comprising the entire extracellular domain of MAdCAM-1 fused to the Fc-mutated IgG1 constant region, was sequenced across the entire MAdCAM-1 portion, confirming proper fusion of segments and the absence of PCR induced mutations.

For initial testing, the construct was transiently transfected onto monolayers of 5 X 10^7 COS cells in 1 ml of RPMI buffer (no serum) and 25 μg of plasmid using electroporation with a Biorad Gene Pulser under standard conditions (960 μF, 250 V). 72-96 hours after transfection, supernatants were harvested, passaged through 0.45 μ filters and stored at 4°C in the presence of 0.05% sodium azide. Production of chimera was confirmed by a sandwich ELISA, using an anti-human IgG1 antibody as capture antibody and the same antibody, which was conjugated to alkaline phosphatase as second antibody for detection. Irrelevant control antibody (having an identical constant region) was used as a standard. The chimera was also analyzed by Western blotting using an anti-human MAdCAM-1 monoclonal antibody, and was found to run at approximately 200 kd, consistent with the size of a homodimer.

<u>Soluble human MAdCAM-Ig chimeras specifically bind α4β7 positive cells</u>

Supernatants from two different transfections were assayed for their ability to stain the T cell line HuT 78, which was previously shown to bind MAdCAM-1 only in the presence of Mn++. Accordingly, each solution used in this assay contained 2 mM Mn++. HuT 78 cells (a human T cell lymphoma line; American Type Culture Collection, Accession

No. ATCC TIB 161) are α4β7-bearing cells. To test the binding specificity of the chimeras, Hut 78 cells were preincubated with either media alone (RPMI 1640 with 2% FCS) or media and 10 μg/ml of the anti-β7 antibody FIB 504. Approximately 100,000 cells were incubated on ice for 15 minutes and then washed with HBSS plus 2% FCS / 2 mM Ca^{++}/ 2 mM Mn^{++}. Cells were then incubated for 20 minutes on ice with media once again, or with supernatants from one of two independent transfections with the construct encoding the chimera comprising the entire extracellular domain of MAdCAM-1 (HuMAdIg21). After washing, cells were then incubated with an anti-human IgG antibody conjugated with phycoerythrin and staining above background was assessed by flow cytometry (FACScan). Only cells incubated with the chimera supernatants stained above background, while preincubation with the β7 MAb reduced this staining to background levels, indicating a specific interaction of the chimera with the α4β7 integrin.

Permanent NSO cell lines secreting human MAdCAM-Ig chimera were selected after transfection by electroporation, by growth in a glutamine free media as previously described (Cockett, M.L., *et al.*, *Bio/Technology*, *8*: 662-667 (1990)). Cloned lines were adapted to growth in spinner culture. Supernatants from three of these cloned lines (samples B-D), and a partially purified chimera (Clone 21, purified by binding to protein A, sample A) were tested for their ability to support adhesion of the B cell line RPMI 8866. Briefly, NEN maxisorb plates were incubated with 100 μl/well of Protein A at 20 μg/ml in carbonate buffer, pH 9.5 overnight at 4°C. Plates were then washed 2X with RPMI 1640 media (no serum). 100 μl of chimera (or serial dilutions in RPMI) were bound to the wells at 37° for 2 hours and then washed once. Wells were then blocked with FCS for 1 hour at 37°C, washed once, and then preincubated with tissue

culture supernatants containing either an anti-human VCAM-1 MAb (2G7) as a control or the anti-human MAdCAM-1 MAb 10G3 (Briskin *et al.*, WO 96/24673, Example 2). 2G7 and 10G3 MAbs were removed before addition of cells. RPMI 8866 cells were fluorescently labeled by preincubation with BCECF-AM stain (BCECF-AM; 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyflourescein, acetoxymethyl ester; Molecular Probes), 100 μl of cells were added to each well (to a final concentration of 10^5 cell/well), and incubated on a rotary shaker for 30 minutes at room temperature. Binding of RPMI 8866 cells to immobilized chimeras was assessed by reading flourescence values using a Fluorescence Concentration Analyzer (IDEXX). Specific binding was demonstrated as only the anti-human MAdCAM-1 MAb could block binding of cells to MAdCAM-Ig chimera (Table 3A).

TABLE 3A

Sample	Anti HuMAd MAbs	Neat	1:2	1:4	1:8	1:16	1:32
A	-	195527	195527	195527	18560	4254	3558
	+	3860	3092	1746	2200	482	564
B	-	195527	195527	195527	195528	52056	2932
	+	6526	3626	3274	2978	1648	1518
C	-	195527	195527	35548	9570	21782	1926
	+	4566	4094	3922	3492	2436	2566
D	-	195527	30840	46852	16270	5474	2656
	+	7350	6794	6020	4510	6548	5122

The α4β7 Positive B Cell Line RPMI 8866 Specifically Binds Soluble Human MAdCAM Ig Chimera. The Human MAdCAM-1 Ig Chimera Clone 21 that was partially purified over protein A (Sample A) or tissue culture supernatants from different NSO clones (Samples B-D) were immobilized on 96 well plates with protein A, and either pre-incubated with an anti-VCAM-1 MAb 2G7 (designated "-" under MAbs) as a negative control or with the anti-human MAdCAM MAb 10G3 ("+"). Purified chimera or supernatants (used either undiluted ("neat") or at serial 1:2 dilutions) was bound to wells via Protein A, and incubated with fluorescently-labeled RPMI 8866 cells on a rotary shaker for 30 minutes at room temperature. After washing with an automated plate washer (EL 404 Microplate Autowasher, BIO-TEK Instruments), bound cells were counted with an automated plate reader (IDEXX). Raw numbers are thus a reflection of numbers of cells bound.

Production and purification of huMAdCAM-1-Ig chimera

NS-0 clone 21-9C10 producing huMAdCAM-1-Ig from HuMAdIG21 was prepared as described above. The clone was grown in glutamine free DMEM, 10% ultra low bovine Ig FCS, 1X GS supplement (Gibco Inc.), (a mixture of non-essential amino acids and nucleosides for addition to glutamine-free basal media), 1 mM pyruvate, 50 units/ml penicillin G and 50 μg/ml streptomycin sulfate. huMAdCAM-1-Ig producing clones were adapted to grow in 1.0 liter spinner flasks at 37°C, 5% CO_2, 95% air and a constant stir rate of 60-70 rpm. Cultures were seeded at a cell density of 1 to 2 x 10^5 cells/ml in 250 mls of the above media and allowed to grow for 48-72 hours before bringing the final volume of the culture to 550 to 600 mls. Spinner flasks were harvested at day 10-11 day centrifugation at 6,000 rpm, 4°C, 25 minutes. The resulting culture supernatants were then 0.2 μ filtered and stored at 4°C.

huMAdCAM-1-Ig culture supernatants were passed over a 20 ml bed volume Protein A column, at equilbrated in PBS, pH 7.2, at 1.5 mls/min flow rate. The column was then washed at 5.0 mls/min with PBS, pH 7.2 at 1.5 mls/min flow rate. The column was then washed at 5.0 mls/min with PBS pH 7.2 and eluted with 0.1 M citrate, pH 3.5 at a flow rate of 5.0 mls/min. 5.0 ml fractions were collected and neutralized immediately with 250 μl/fraction 1.5 M sodium carbonate, pH 11.5. The Protein A column was then equilibrated with PBS, pH 7.2.

The resulting fractions from Protein A purification were analyzed by an anti-human Ig(Fc) (Jackson Research Labs.) ELISA. Peak fractions containing huMAdCAM-1-Ig were concentrated by centrifugation at 6,000 rpm, 4°C with Centricon 30 concentrators (30,000 mw cutoff).

Final product was quantitated by BioRad Protein Assay, analyzed for biological activity in the huMAdCAM-1-Ig/RPMI 8866 Adhesion Assay (see below) and for purity with 4-20%

SDS-PAGE, reduced and nonreduced, by colloidal coomassie blue staining.

Adhesion Assay Protocol

RPMI 8866 cells, a B cell lymphoma expressing α4β7 (a gift from D. Erle; Erle, D.J. *et al.*, *J. Immunol.*, *153*:517-528 (1994); Shyjan *et al.*, *Journal of Immunology*, *1996*:2851 (1996) were flourescently labeled (labeling protocol provided below) using BCECF (Molecular probes #B-1170). Cells were resuspended in an assay buffer consisting of Hanks Balanced Salt Solution (HBSS) supplemented with fetal calf serum (FCS) at a final concentration of 2%, HEPES, pH 7.3 at 25 mM. All reagents in this buffer were supplied from Gibco/BRL.

All assays were performed in 96 well strip well plates from Costar (E.I.A./R.I.A. Strip plate-8 Cat. #2581. Protein A purified human MAdCAM-1-Ig chimera was suspended in carbonate buffer (0.2 M $NaHCO_3$ and 0.8 M Na_2CO_3, pH 9.5) at a concentration of 200 ng/ml and 50 µl was added to each well for a plating concentration of 10 ng/well. The plates were incubated at 37°C for one hour (or at 4° overnight) and washed once in the automatic plate washer (50 mM Tris HCl, .14 M NaCl and 2.0 mM $MnCl_2$ at pH 7.2) (Bio-Tek EL 404 microplate autowasher). Wells were then blocked with PBS (phosphate buffered saline) and 10% FCS (100 µl/well) for one hour at 37° followed by the same wash step as described.

Cells were labeled with BCECF-AM (Molecular Probes, Cat. No. B-1170) at a concentration of 2 µl/ml to a cell suspension of 4 x 10^6 cells/ml in PBS. The cells were incubated in a 37°C water bath for 30 minutes. Labeled cells were centrifuged at 700 RPM for 10 minutes, resuspended in assay buffer and the centrifugation step was repeated. The cells were resuspended at a final concentration of 2.5 x 10^6 cells/ml.

For assays, compounds were diluted as follows in a 96 well polypropylene plate. First, a stock solution of each compound was prepared by dissolving compound in 100% DMSO at 100X final concentration to be tested. 10 μl of the stock was added to 90 μl water for the first test dilution. 50 μl of the first test dilution was added to 50 μl of 10% DMSO in water for the second test dilution. 50 μl of the second test dilution was added to 50 μl of 10% DMSO in water for the third test dilution. 50 μl of the third test dilution was added to 50 μl of 10% DMSO in water for the fourth test dilution, and so on, until six test dilutions were prepared.

A stock solution of 5mM positive control peptide (L783-1A) was made in 100% DMSO, and subsequently diluted in water 1:10 (10μl of 5 mM stock plus 90μl of water) to yield a concentration of .5mM. This dilution was then further diluted 1:2 with 10% DMSO (50μl of the compound and 50μl of 10% DMSO) for a concentration of 250μM. These 1:2 dilutions were continued seriously until six dilutions were achieved. These dilutions represent 10X stocks as shown in the assay set up below:

The assay was set up by adding materials to huMAdCAM-1-Ig-coated plates as follows:

1) 130μl/well of assay buffer
2) 20μl/well of diluted compounds or 10% DMSO (control) or diluted positive control peptide.
3) 50μl of BCECF-AM-labeled RPMI 8866 cells

In this example, the final concentration of cells was 1.25×10^6 cells/well and the final concentration of compound ranged in a six point assay from 50μm to 1.06μm.

The plates were wrapped in foil and incubated for 30 minutes at room temperature on a rotary shaker at 45 RPM.

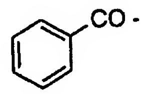
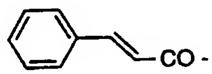
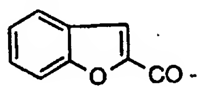
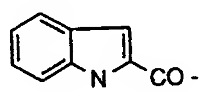
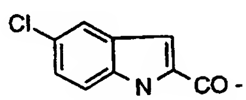
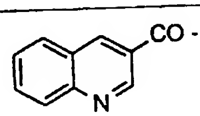
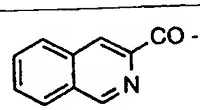
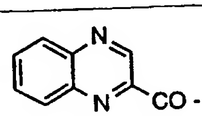
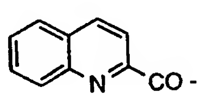
Plates were washed twice (50 mM Tris Hcl, .14 mM NaCl and 2.0 mM $MnCl_2$ at pH 7.2) on the microplate autowasher plate washer set as follows:

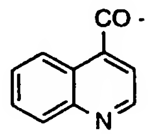
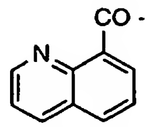
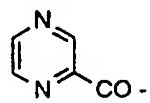
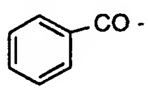
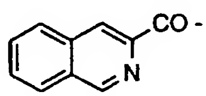
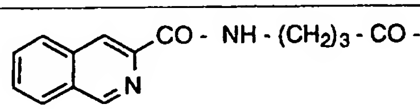
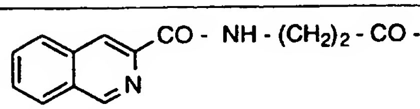
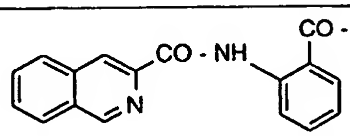
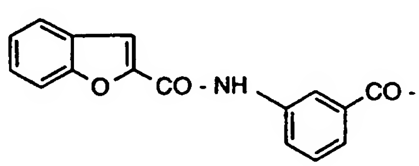
Wash Volume = 500ml
Wash Cycle = 2X
Soak Time = 0
Wash Depth = 80
Aspirate After Last Wash
Shake Time = 0

Plates were read in an IDEX flouresecent plate reader set at excite at 485nm and read at 535nm.

Data was collected in microsoft excel in a format for automatic IC 50 determinations. The IC 50 is the fluorescence level that is 50% of maximum binding. Results are shown in Tables 4-7.

In Table 4, Example No. 1478 is represented by SEQ ID NO: 71; Example No. 783 is represented by SEQ ID NO: 72; Example No. 1492 is represented by SEQ ID NO: 73; Example No. 1487 is represented by SEQ ID NO: 74; Example No. 1490 is represented by SEQ ID NO: 75; Example No. 1275 is represented by SEQ ID NO: 76; Example No. 1276 is represented by SEQ ID NO: 77; Example No. 1282 is represented by SEQ ID NO: 78; Example No. 1481 is represented by SEQ ID NO: 79; Example No. 1482 is represented by SEQ ID NO: 80; Example No. 1486 is represented by SEQ ID NO: 81; Example No. 1491 is represented by SEQ ID NO: 82.

Table 4.	R_1-Leu-Asp-Thr-Ser-Leu				
L #	R_1	Method Prep	Mass Calc.	Mass Found	hMAdCAM IC50
1478		C	647	658	11.2
783		C	673	701	10
1492		C	687	715	2.1
1487		C	686	690	3
1490		C	720	724	1.7
1275		C	698	726	3.5
1276		C	698	703	2.7
1282		C	699	706	4
1481		C	698	705	3.2

1482		C	698	700	2.2
1486		C	698	705	1.4
1491		C	649	658	3.4
Table 5.	**R_1-Leu-Asp-Thr**				
L #	**R_1**	**Method Prep**	**Mass Calc.**	**Mass Found**	**hMAdCAM IC50**
1496		C	448	503	8.2
1495		C	499	501	3.1
2277		C	584	587	1.7
2278		C	570	575	0.4
2273		C	618	620	1.2
2271		C	607	601	0.9

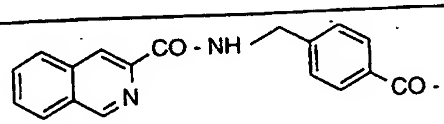
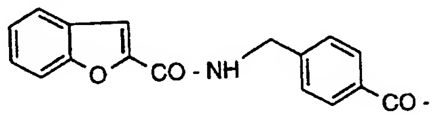
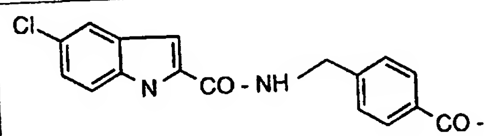
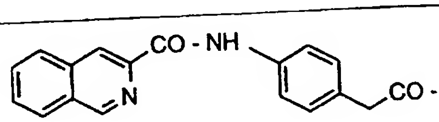
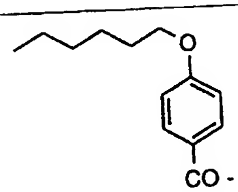
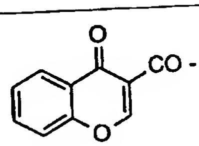
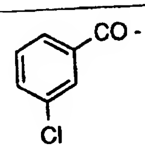
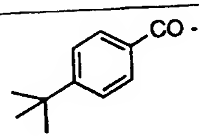
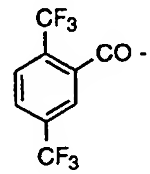
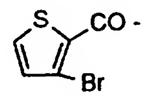
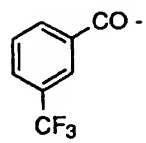
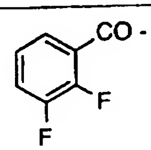
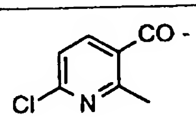
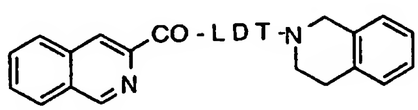
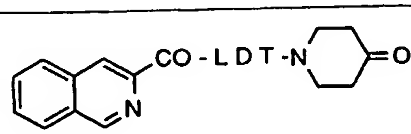
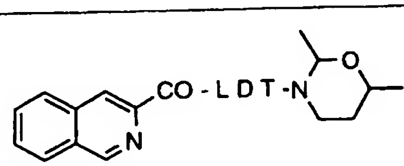
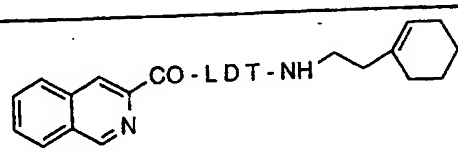
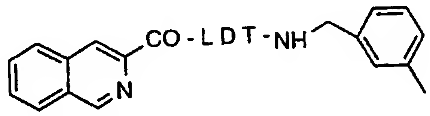
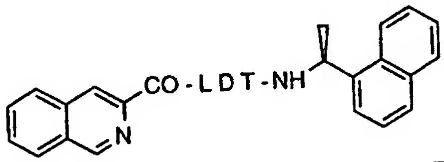
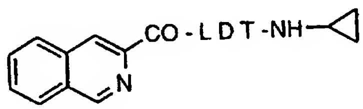
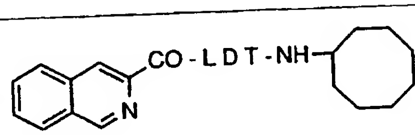
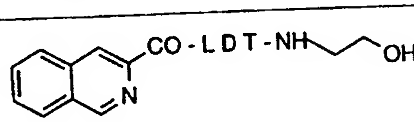
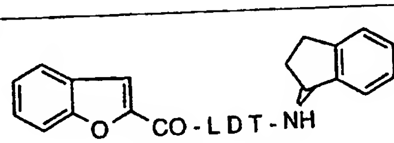
2261		C	632	633	0.86
2263		C	621	622	1.3
2262		C	654	679	1.5
2279		C	632	636	1

Table 6. **R_1-Leu-Asp-Thr**

Example	R_1	Method Prep	Mass Calc.	Mass Found	hMAdCAM IC50
1		C	550	550	0.58
2		C	518	518	0.73
3		C	484	484	1.4
4		C	506	506	1.1

5		C	493	493	1.5
6		C	535	534	2.6
7		C	518	518	2.8
8		C	486	486	2
9		C	499	499	1.1
Table 7.	**R_1-Leu-Asp-Thr-R_2**				
Example	**R_1-Leu-Asp-Thr-R_2**	**Method Prep**	**Mass Calc.**	**Mass Found**	**hMAdCAM IC50**
1		F	617	617	0.78
2		F	583	583	0.94
3		F	599	599	0.97

4		F	609	609	0.64
5		F	605	605	0.81
6		F	655	655	0.97
7		F	541	541	3.8
8		F	611	611	1.3
9		F	545	545	1.5
10		F	606	617	1.5

Equivalents

Those skilled in the art will be able to recognize, or be able to ascertain, using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

CLAIMS

What is claimed is.

1. A compound represented by the following structural formula:

$$R^1\text{-}X\text{-}Y'\text{-}Z\text{-}R^2$$

wherein:

Y' is a pentapeptide $[AA]_1$-$[AA]_2$-$[AA]_3$-$[AA]_4$-$[AA]_5$ having the sequence Leu-Asp-Thr-Ser-Leu (SEQ ID NO: 1) with the proviso any single one of $[AA]_1$, $[AA]_2$, $[AA]_3$, $[AA]_4$ or $[AA]_5$ can vary, being any naturally occurring amino acid;

X and Z are independently chosen from the group consisting of a covalent bond, an amino acid or a peptide, wherein each amino acid in X and Z is independently selected from the group of naturally occurring amino acids;

R^1 is R^3-CO-;

R^2 is $-NR^4R^5$;

R^3 is selected from the group consisting of a lower alkyl group, a substituted lower alkyl group, an aryl group, a substituted aryl group, a heteroaryl group and a substituted heteroaryl group;

R^4 and R^5 are each independently selected from the group consisting of hydrogen, a lower alkyl group, a substituted lower alkyl group, an aryl group, a substituted aryl group, a heteroaryl group and a substituted heteroaryl group, wherein:

1) R^4 and R^5 are not both -H; and
2) taken together, R^4 and R^5 can form a heterocyclic ring;

taken together, X, Y' and Z form a peptide containing no more than about fifteen amino acids; and

wherein optionally the peptide formed from X, Y' and Z is cyclized.

2. The compound of Claim 1 wherein R^3 is selected from the group consisting of triphenylmethyl, diphenylmethyl, 3,5-diphenylphenyl, 2-furanyl, 3-furanyl, 9-xanthenemethyl, 2,2,2-triphenylethyl, 2-anthracene, methyl, cyclopentyl, 2-indolyl, 2-indanyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl, 3-benzothienyl, cyclohexyl, 5-phenylpentyl, 4-isobutyl-α-methylphenylmethyl, 4-biphenylmethyl, α-naphthylmethyl, 4-heptylphenyl, phenylmethyl, *trans* 2-phenylethenyl and 2,2,3,3-tetramethylcyclopropyl.

3. The compound of Claim 2 wherein R^4 and R^5 are each independently selected from the group consisting of -H, 2-hydroxyethyl, benzyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl, 3-benzothienyl, $-CH_2$-2-thienyl, $-CH_2$-3-thienyl, $-CH_2$-2-furanyl, $-CH_2$-3-furanyl, 3,4-dimethoxybenzyl, and isopentyl.

4. The compound of Claim 3 wherein Y' is Leu-Asp-Thr-Ser-Leu (SEQ ID NO: 1).

5. The compound of Claim 1 wherein:

R^3 is selected from the group consisting of diphenylmethyl, triphenylmethyl, *trans* 2-phenyl-ethylenyl, 2-phenyl-ethynyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl and 3-benzothienyl;

R^4 is selected from the group consisting of 2-hydroxyethyl, benzyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl, 3-benzothienyl, $-CH_2$-2-thienyl, $-CH_2$-3-thienyl, $-CH_2$-2-furanyl, $-CH_2$-3-furanyl; and

R^5 is -H.

6. The compound of Claim 5 wherein X and Z are each a covalent bond.

7. The compound of Claim 1 wherein the peptide formed from X, Y' and Z is cyclized.

8. A compound represented by the following structural formula:

$$R^1\text{-X-Y'-Z-}R^2$$

wherein:

Y' is a pentapeptide $[AA]_1$-$[AA]_2$-$[AA]_3$-$[AA]_4$-$[AA]_5$ having the sequence Leu-Asp-Thr-Ser-Leu (SEQ ID NO: 1) with the proviso that any single one of $[AA_1]$, $[AA_2]$, $[AA_3]$, $[AA_4]$ and $[AA_5]$ can vary, wherein

$[AA]_1$ is selected from the group consisting of leucine, valine, isoleucine, alanine, glycine, phenylalanine and N-methylleucine;

$[AA]_2$ is selected from the group consisting of aspartic acid, glutamic acid, phenylalanine and tyrosine;

$[AA]_3$ is selected from the group consisting of threonine, serine, valine, proline and 4-hydroxyproline;

$[AA]_4$ is selected from the group consisting of serine, cysteine and threonine; and

$[AA]_5$ is selected from the group consisting of alanine, valine, leucine, isoleucine, alanine, glycine, phenylalanine and N-methylleucine;

X and Z are independently chosen from the group consisting of a covalent bond, an amino acid or a peptide, wherein each amino acid in X and Z is independently selected from the group of naturally occurring amino acids;

R^1 is R^3-CO-;

R^2 is $-NR^4R^5$;

R^3 is selected from the group consisting of a lower alkyl group, a substituted lower alkyl group, an aryl group, a substituted aryl group, a heteroaryl group and a substituted heteroaryl group;

R^4 and R^5 are each independently selected from the group consisting of hydrogen, a lower alkyl group, a substituted lower alkyl group, an aryl group, a substituted aryl group, a heteroaryl group and a substituted heteroaryl group, wherein:

1) R^4 and R^5 are not both -H; and
2) taken together, R^4 and R^5 can form a heterocyclic ring;

taken together, X, Y' and Z form a peptide containing no more than about fifteen amino acids; and

wherein optionally the peptide formed from X, Y' and Z is cyclized.

9. A compound represented by the following structural formula:

$$R^1\text{-}Y'\text{-}R^2$$

wherein:

Y' is a pentapeptide $[AA]_1$-$[AA]_2$-$[AA]_3$-$[AA]_4$-$[AA]_5$ having the sequence Leu-Asp-Thr-Ser-Leu (SEQ ID NO: 1) with the proviso that any single one of $[AA]_1$, $[AA]_2$, $[AA]_3$, $[AA]_4$ or $[AA]_5$ can vary, being any naturally occurring amino acid, wherein:

R^1 is R^3-CO-;

R^2 is $-NR^4R^5$;

R^3 is selected from the group consisting of a lower alkyl group, a substituted lower alkyl group, an aryl group, a substituted aryl group, a heteroaryl group and a substituted heteroaryl group; and

R^4 and R^5 are each independently selected from the group consisting of hydrogen, a lower alkyl group, a substituted lower alkyl group, an aryl group, a

substituted aryl group, a heteroaryl group and a substituted heteroaryl group, wherein:

1) R^4 and R^5 are not both -H; and
2) taken together, R^4 and R^5 can form a heterocyclic ring; and

wherein optionally Y is cyclized.

10. The compound of Claim 9 wherein Y' has the sequence Leu-Asp-Thr-Ser-Leu (SEQ ID NO: 1).

11. The compound of Claim 10 wherein R^3 is selected from the group consisting of monocyclic and bicyclic nitrogen-containing heteroaromatic groups, vinyl groups substituted with substituted and unsubstituted aryl and heteroaryl groups, polycarbocyclic aromatic hydrocarbons and oxygen-containing polycyclic aromatic hydrocarbons.

12. The compound of Claim 10 wherein R^3 is selected from the group consisting of a quinolinyl group, an isoquinolinyl group, an indolyl group, a quinoxalinyl group, a cinnolinyl group, a pyrazinyl group, a styryl group, a stilbyl group, (3-pyridyl)-CH=CH-, a naphthyl group, an anthracyl group, a xanthanyl group, a benzopyranone group and a benzofuranyl group.

13. A compound represented by the following structural formula:

$$R^1\text{-}X\text{-}Y'\text{-}Z\text{-}R^2$$

wherein:

Y' is a tripeptide $[AA]_1$-$[AA]_2$-$[AA]_3$ having the sequence Leu-Asp-Thr;

X and Z are independently chosen from the group consisting of a covalent bond, an amino acid or a peptide, wherein each amino acid in X and Z is

independently selected from the group of naturally occurring amino acids;

R^1 is R^3-CO-;

R^2 is $-NR^4R^5$;

R^3 is selected from the group consisting of a lower alkyl, substituted lower alkyl, aryl, substituted aryl, heteroaryl and substituted heteroaryl; and

R^4 and R^5 are each independently selected from the group consisting of hydrogen, a lower alkyl group, a substituted lower alkyl group, an aryl group, a substituted aryl group, a heteroaryl group and a substituted heteroaryl group, wherein:

1) R^4 and R^5 are not both -H; and
2) taken together, R^4 and R^5 can form a heterocyclic ring; and

taken together, X, Y' and Z form a peptide containing no more than about fifteen amino acids; and

wherein optionally the peptide formed from X, Y' and Z is cyclized.

14. The compound of Claim 13 wherein R^3 is selected from the group consisting of triphenylmethyl, diphenylmethyl, 3,5-diphenylphenyl, 2-furanyl, 3-furanyl, 9-xanthenemethyl, 2,2,2-triphenylethyl, 2-anthracene, methyl, cyclopentyl, 2-indolyl, 2-indanyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl, 3-benzothienyl, cyclohexyl, 5-phenylpentyl, 4-isobutyl-α-methylphenylmethyl, 4-biphenylmethyl, α-naphthylmethyl, 4-heptylphenyl, phenylmethyl, *trans* 2-phenylethenyl and 2,2,3,3-tetramethylcyclopropyl.

15. The compound of Claim 14 wherein R^4 and R^5 are each independently selected from the group consisting of -H, 2-hydroxyethyl, benzyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl, 3-benzothienyl, $-CH_2$-2-thienyl, $-CH_2$-3-

thienyl, $-CH_2$-2-furanyl, $-CH_2$-3-furanyl, 3,4-dimethoxybenzyl, and isopentyl.

16. The compound of Claim 13 wherein:

R^3 is selected from the group consisting of diphenylmethyl, triphenylmethyl, *trans* 2-phenyl-ethylenyl, 2-phenyl-ethynyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl and 3-benzothienyl;

R^4 is selected from the group consisting of 2-hydroxyethyl, benzyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl, 3-benzothienyl, $-CH_2$-2-thienyl, $-CH_2$-3-thienyl, $-CH_2$-2-furanyl, $-CH_2$-3-furanyl; and

R^5 is -H.

17. The compound of Claim 13 wherein the peptide formed from X, Y' and Z is cyclized.

18. A compound represented by the following structural formula:

$$R^1\text{-}Y'\text{-}R^2$$

wherein:

Y' is a tripeptide $[AA]_1$-$[AA]_2$-$[AA]_3$ having the sequence Leu-Asp-Thr;

R^1 is R^3-CO-;

R^2 is $-NR^4R^5$;

R^3 is selected from the group consisting of a lower alkyl, substituted lower alkyl, aryl, substituted aryl, heteroaryl and substituted heteroaryl; and

R^4 and R^5 are each independently selected from the group consisting of hydrogen, a lower alkyl group, a substituted lower alkyl group, an aryl group, a substituted aryl group, a heteroaryl group and a substituted heteroaryl group, wherein:

1) R^4 and R^5 are not both -H; and

2) taken together, R^4 and R^5 can form a heterocyclic ring.

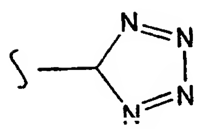
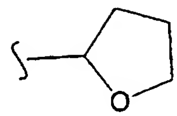
19. The compound of Claim 18 wherein R^3 is selected from the group consisting of phenyl, substituted phenyl, thienyl, substituted thienyl, indolyl, substituted indolyl, pyrimidyl, substituted pyrimidyl, benzofuranyl, substituted benzofuranyl, quinolinyl, substituted quinolinyl, isoquinolinyl, substituted isoquinolinyl, benzopyranone groups, substituted benzopyranone groups and 3-isoquinolinyl-CO-NH-$(CH_2)_x$-, wherein x is an integer from 1-4.

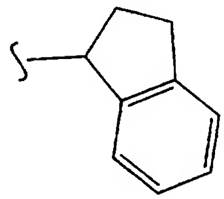
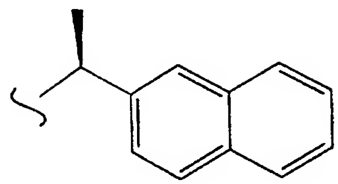
20. The compound of Claim 19 wherein:

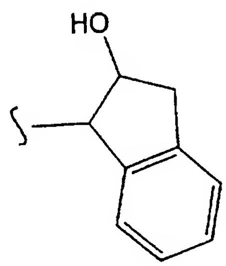
R^3 is 3-isoquinolinyl or 2-benzofuranyl;

R^4 is -H; and

R^5 is benzyl, substituted benzyl, phenethyl, substituted phenethyl, phenpropyl, substituted phenpropyl, heteroaryl-CH_2-, substituted heteroaryl-CH_2-, lower alkyl, substituted lower alkyl, cycloalkyl and a group represented by one of the following structural formulas:

 ,  ,

 , 

and  .

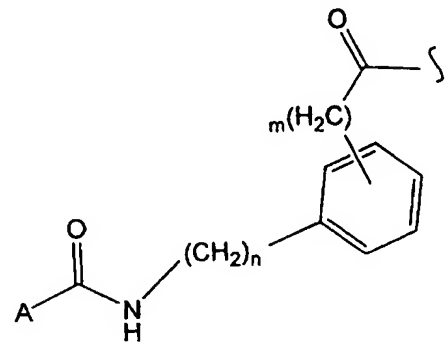
21. The compound of Claim 19 wherein:

R^3 is 3-isoquinolinyl or 2-benzofuranyl; and

R^4 and R^5, taken together, form a hetercyclic ring selected from the group consisting of pyrrolidinyl, substituted pyrrolidinyl, indoline, isomers of indoline, substituted indoline, substituted isomers of indoline, tetrahydroisoquinoline, substituted tetrahydroisoquinoline, tetrahydroquinoline, substituted tetrahydroquinoline, piperidone, substituted piperidone, piperidine, substituted

piperidines, tetrahydro-oxazines and substituted tetrahydro-oxazines.

22. The compound of Claim 18 wherein R^1 is represented by the following structural formula:



wherein:

A is selected from the group consisting of an aryl group, a substituted aryl group, a heteroaryl group and a substituted heteroarylgroup; and

n and m are each independently zero or one.

23. A compound represented by the following structural formula:

$$R^1\text{-X-Y'-Z-}R^2$$

wherein:

Y' is a dipeptide $[AA]_1$-$[AA]_2$ having the sequence Asp-Thr;

X and Z are independently chosen from the group consisting of a covalent bond, an amino acid or a peptide, wherein each amino acid in X and Z is independently selected from the group of naturally occurring amino acids;

R^1 is R^3-CO-;

R^2 is $-NR^4R^5$;

R^3 is selected from the group consisting of a lower alkyl, substituted lower alkyl, aryl, substituted aryl, heteroaryl and substituted heteroaryl; and

R^4 and R^5 are each independently selected from the group consisting of hydrogen, a lower alkyl group, a substituted lower alkyl group, an aryl group, a substituted aryl group, a heteroaryl group and a substituted heteroaryl group, wherein:

1) R^4 and R^5 are not both -H; and
2) taken together, R^4 and R^5 can form a heterocyclic ring; and

taken together, X, Y' and Z form a peptide containing no more than about fifteen amino acids; and

wherein optionally the peptide formed from X, Y' and Z is cyclized with the proviso that, if the peptide formed from X, Y' and Z is cyclized, the nitrogen at the N-terminus of Y' is not bonded to a glycine or a sarcosine.

24. The compound of Claim 23 wherein R^3 is selected from the group consisting of triphenylmethyl, diphenylmethyl, 3,5-diphenylphenyl, 2-furanyl, 3-furanyl, 9-xanthenemethyl, 2,2,2-triphenylethyl, 2-anthracene, methyl, cyclopentyl, 2-indolyl, 2-indanyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl, 3-benzothienyl, cyclohexyl, 5-phenylpentyl, 4-isobutyl-α-methylphenylmethyl, 4-biphenylmethyl, α-naphthylmethyl, 4-heptylphenyl, phenylmethyl, *trans* 2-phenylethenyl and 2,2,3,3-tetramethylcyclopropyl.

25. The compound of Claim 24 wherein R^4 and R^5 are each independently selected from the group consisting of -H, 2-hydroxyethyl, benzyl, 2-benzofuranyl, 3-benzofuranyl,

2-benzothienyl, 3-benzothienyl, $-CH_2$-2-thienyl, $-CH_2$-3-thienyl, $-CH_2$-2-furanyl, $-CH_2$-3-furanyl, 3,4-dimethoxybenzyl, and isopentyl.

26. The compound of Claim 23 wherein:

R^3 is selected from the group consisting of R^3 is selected from the group consisting of diphenylmethyl, triphenylmethyl, *trans* 2-phenyl-ethylenyl, 2-phenyl-ethynyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl and 3-benzothienyl;

R^4 is selected from the group consisting of 2-hydroxyethyl, benzyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl, 3-benzothienyl, $-CH_2$-2-thienyl, $-CH_2$-3-thienyl, $-CH_2$-2-furanyl, $-CH_2$-3-furanyl; and

R^5 is -H.

27. The compound of Claim 26 wherein X and Z are each a covalent bond.

28. The compound of Claim 23 wherein the peptide formed from X, Y' and Z is cyclized.

29. A method of treating an individual suffering from a disease associated with leukocyte infiltration of tissues expressing the molecule MAdCAM-1, comprising administering a therapeutically effective amount of an inhibitor represented by the following structural formula:

$$R^1\text{-}X\text{-}Y\text{-}Z\text{-}R^2$$

wherein:

Y is a pentapeptide $[AA]_1$-$[AA]_2$-$[AA]_3$-$[AA]_4$-$[AA]_5$ wherein:

$[AA]_1$ is selected from the group consisting of leucine, cysteine, aspartic acid, glutamic acid, isoleucine, alanine, valine, phenylalanine,

glycine, N-methylleucine, serine, threonine, ornithine and lysine;

$[AA]_2$ is selected from the group consisting of aspartic acid, glutamic acid, phenylalanine and tyrosine;

$[AA]_3$ is selected from the group consisting of threonine, serine, valine, proline and 4-hydroxyproline;

$[AA]_4$ is selected from the group consisting of serine, cysteine, aspartic acid, glutamic acid, proline, 4-hydroxyproline, threonine, valine, isoleucine, alanine, glycine, ornithine and lysine; and

$[AA]_5$ is selected from the group consisting of leucine, isoleucine, N-methylleucine, threonine, ornithine, serine, valine, alanine, glycine, phenylalanine, cysteine, aspartic acid, glutamic acid and lysine;

X and Z are independently chosen from the group consisting of a covalent bond, an amino acid or a peptide, wherein each amino acid in X and Z is independently selected from the group of naturally occurring amino acids;

R^1 is R^3-CO-;

R^2 is $-NR^4R^5$;

R^3 is selected from the group consisting of a lower alkyl, substituted lower alkyl, aryl, substituted aryl, heteroaryl and substituted heteroaryl; and

R^4 and R^5 are each independently selected from the group consisting of hydrogen, a lower alkyl group, a substituted lower alkyl group, an aryl group, a substituted aryl group, a heteroaryl group and a substituted heteroaryl group, wherein:

1) R^4 and R^5 are not both -H; and

2) taken together, R^4 and R^5 can form a heterocyclic ring; and

taken together, X, Y and Z form a peptide containing no more than about fifteen amino acids; and wherein:

optionally the peptide formed by X, Y and Z is cyclized; and

an arginine or an arginine derivative is not bonded to the nitrogen at the N-terminus of Y.

30. The method of Claim 29 wherein Y is a pentapeptide $[AA]_1$-$[AA]_2$-$[AA]_3$-$[AA]_4$-$[AA]_5$ having the sequence Leu-Asp-Thr-Ser-Leu (SEQ ID NO: 1) with the proviso that any single one of $[AA]_1$, $[AA]_2$, $[AA]_3$, $[AA]_4$ or $[AA]_5$ can vary, being any naturally occurring amino acid.

31. The method of Claim 30 wherein R^3 is selected from the group consisting of triphenylmethyl, diphenylmethyl, 3,5-diphenylphenyl, 2-furanyl, 3-furanyl, 9-xanthenemethyl, 2,2,2-triphenylethyl, 2-anthracene, methyl, cyclopentyl, 2-indolyl, 2-indanyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl, 3-benzothienyl, cyclohexyl, 5-phenylpentyl, 4-isobutyl-α-methylphenylmethyl, 4-biphenylmethyl, α-naphthylmethyl, 4-heptylphenyl, phenylmethyl, *trans* 2-phenylethenyl and 2,2,3,3-tetramethylcyclopropyl.

32. The method of Claim 31 wherein R^4 and R^5 are each independently selected from the group consisting of -H, 2-hydroxyethyl, benzyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl, 3-benzothienyl, $-CH_2$-2-thienyl, $-CH_2$-3-thienyl, $-CH_2$-2-furanyl, $-CH_2$-3-furanyl, 3,4-dimethoxybenzyl, and isopentyl.

33. The method of Claim 29 wherein:

$[AA]_1$ is selected from the group consisting of leucine, valine, isoleucine, alanine, glycine, phenylalanine and N-methylleucine;

$[AA]_2$ is selected from the group consisting of aspartic acid, glutamic acid, phenylalanine and tyrosine;

$[AA]_3$ is selected from the group consisting of threonine, serine, valine, proline and 4-hydroxyproline;

$[AA]_4$ is selected from the group consisting of serine, cysteine and threonine; and

$[AA]_5$ is selected from the group consisting of alanine, valine, leucine, isoleucine, alanine, glycine, phenylalanine and N-methylleucine.

34. The method of Claim 33 wherein Y is Leu-Asp-Thr-Ser-Leu (SEQ ID NO: 1).

35. The method of Claim 29 wherein:

R^3 is selected from the group consisting of diphenylmethyl, triphenylmethyl, *trans* 2-phenyl-ethylenyl, 2-phenyl-ethynyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl and 3-benzothienyl;

R^4 is selected from the group consisting of 2-hydroxyethyl, benzyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl, 3-benzothienyl, $-CH_2$-2-thienyl, $-CH_2$-3-thienyl, $-CH_2$-2-furanyl, $-CH_2$-3-furanyl;

and R^5 is -H.

36. The method of Claim 29 wherein the peptide formed from X, Y and Z is cyclized.

37. A method of treating an individual suffering from a disease associated with leukocyte infiltration of

tissues expressing the molecule MAdCAM-1, comprising administering a therapeutically effective amount of an inhibitor represented by the following structural formula:

$$R^1\text{-}Y'\text{-}R^2$$

wherein:

Y' is a pentapeptide $[AA]_1$-$[AA]_2$-$[AA]_3$-$[AA]_4$-$[AA]_5$ having the sequence Leu-Asp-Thr-Ser-Leu (SEQ ID NO: 1) with the proviso that any single one of $[AA]_1$, $[AA]_2$, $[AA]_3$, $[AA]_4$ or $[AA]_5$ can vary, being any naturally occurring amino acid, wherein:

R^1 is R^3-CO-;

R^2 is $-NR^4R^5$;

R^3 is selected from the group consisting of a lower alkyl group, a substituted lower alkyl group, an aryl group, a substituted aryl group, a heteroaryl group and a substituted heteroaryl group; and

R^4 and R^5 are each independently selected from the group consisting of hydrogen, a lower alkyl group, a substituted lower alkyl group, an aryl group, a substituted aryl group, a heteroaryl group and a substituted heteroaryl group, wherein:

1) R^4 and R^5 are not both -H; and
2) taken together, R^4 and R^5 can form a heterocyclic ring; and

wherein optionally Y is cyclized.

38. The method of Claim 37 wherein Y' has the sequence Leu-Asp-Thr-Ser-Leu (SEQ ID NO: 1).

39. The method of Claim 38 wherein R^3 is selected from the group consisting of a monocyclic and bicyclic nitrogen-containing heteroaromatic groups, vinyl groups substituted with substituted and unsubstituted aryl and heteroaryl groups, polycarbocyclic aromatic

hydrocarbons and oxygen-containing polycyclic aromatic hydrocarbons.

40. The method of Claim 39 wherein R^3 is selected from the group consisting of a quinolinyl group, an isoquinolinyl group, an indolyl group, a quinoxalinyl group, a cinnolinyl group, a pyrazinyl group, a styryl group, a stilbyl group, (3-pyridyl)-CH=CH-, a naphthyl group, an anthracyl group, a xanthanyl group, a benzopyranone group and a benzofuranyl group.

41. The method of Claim 29 wherein the disease is selected from the group consisting of inflammatory bowel disease and insulin-dependent diabetes mellitus.

42. A method of treating an individual suffering from a disease associated with leukocyte infiltration of tissues expressing the molecule MAdCAM-1, comprising administering a therapeutically effective amount of an inhibitor represented by the following structural formula:

$$R^1\text{-}X\text{-}Y'\text{-}\dot{Z}\text{-}R^2$$

wherein:

Y' is a tripeptide $[AA]_1$-$[AA]_2$-$[AA]_3$ having the sequence Leu-Asp-Thr;

X and Z are independently chosen from the group consisting of a covalent bond, an amino acid or a peptide, wherein each amino acid in X and Z is independently selected from the group of naturally occurring amino acids;

R^1 is R^3-CO-;

R^2 is $-NR^4R^5$;

R^3 is selected from the group consisting of a lower alkyl, substituted lower alkyl, aryl, substituted aryl, heteroaryl and substituted heteroaryl; and

R^4 and R^5 are each independently selected from the group consisting of hydrogen, a lower alkyl group, a substituted lower alkyl group, an aryl group, a substituted aryl group, a heteroaryl group and a substituted heteroaryl group, wherein:

1) R^4 and R^5 are not both -H; and
2) taken together, R^4 and R^5 can form a heterocyclic ring; and

taken together, X, Y' and Z form a peptide containing no more than about fifteen amino acids; and

wherein optionally the peptide formed from X, Y' and Z is cyclized.

43. The method of Claim 42 wherein R^3 is selected from the group consisting of triphenylmethyl, diphenylmethyl, 3,5-diphenylphenyl, 2-furanyl, 3-furanyl, 9-xanthenemethyl, 2,2,2-triphenylethyl, 2-anthracene, methyl, cyclopentyl, 2-indolyl, 2-indanyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl, 3-benzothienyl, cyclohexyl, 5-phenylpentyl, 4-isobutyl-α-methylphenylmethyl, 4-biphenylmethyl, α-naphthylmethyl, 4-heptylphenyl, phenylmethyl, *trans* 2-phenylethenyl and 2,2,3,3-tetramethylcyclopropyl.

44. The method of Claim 43 wherein R^4 and R^5 are each independently selected from the group consisting of -H, 2-hydroxyethyl, benzyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl, 3-benzothienyl, $-CH_2$-2-thienyl, $-CH_2$-3-thienyl, $-CH_2$-2-furanyl, $-CH_2$-3-furanyl, 3,4-dimethoxybenzyl, and isopentyl.

45. The method of Claim 42 wherein:

R^3 is selected from the group consisting of diphenylmethyl, triphenylmethyl, *trans*

2-phenyl-ethylenyl, 2-phenyl-ethynyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl and 3-benzothienyl;

R^4 is selected from the group consisting of 2-hydroxyethyl, benzyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl, 3-benzothienyl, $-CH_2$-2-thienyl, $-CH_2$-3-thienyl, $-CH_2$-2-furanyl, $-CH_2$-3-furanyl;

and R^5 is -H.

46. The method of Claim 43 wherein the peptide formed from X, Y' and Z is cyclized.

47. The method of Claim 42 wherein the disease is selected from the group consisting of inflammatory bowel disease and insulin-dependent diabetes mellitus.

48. A method of treating an individual suffering from a disease associated with leukocyte infiltration of tissues expressing the molecule MAdCAM-1, comprising administering a therapeutically effective amount of an inhibitor represented by the following structural formula:

$$R^1\text{-}Y'\text{-}R^2$$

wherein:

Y' is a tripeptide $[AA]_1$-$[AA]_2$-$[AA]_3$ having the sequence Leu-Asp-Thr;

R^1 is R^3-CO-;

R^2 is $-NR^4R^5$;

R^3 is selected from the group consisting of a lower alkyl, substituted lower alkyl, aryl, substituted aryl, heteroaryl and substituted heteroaryl; and

R^4 and R^5 are each independently selected from the group consisting of hydrogen, a lower alkyl group, a substituted lower alkyl group, an aryl group, a substituted aryl group, a heteroaryl group and a substituted heteroaryl group, wherein:

1) R^4 and R^5 are not both -H; and
2) taken together, R^4 and R^5 can form a heterocyclic ring.

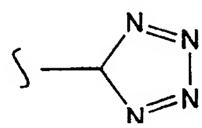
49. The method of Claim 48 wherein R^3 is selected from the group consisting of phenyl, substituted phenyl, thienyl, substituted thienyl, indolyl, substituted indolyl, pyrimidyl, substituted pyrimidyl, benzofuranyl, substituted benzofuranyl, quinolinyl, substituted quinolinyl, isoquinolinyl, substituted isoquinolinyl, benzopyranone groups, substituted benzopyranone groups, and 3-isoquinolinyl-CO-NH-$(CH_2)_x$-, wherein x is an integer from 1-4.

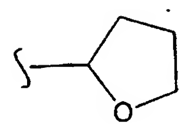
50. The method of Claim 49 wherein:

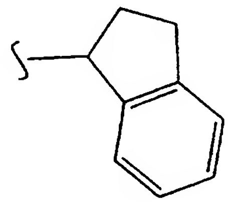
R^3 is 3-isoquinolinyl or 2-benzofuranyl;

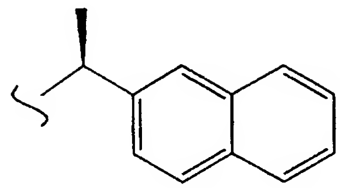
R^4 is -H; and

R^5 is benzyl, substituted benzyl, phenethyl, substituted phenethyl, phenpropyl, substituted phenpropyl, heteroaryl-CH_2-, substituted heteroaryl-CH_2-, lower alkyl, substituted lower alkyl, cycloalkyl, substituted cycloalkyl and a group represented by one of the following structural formulas:

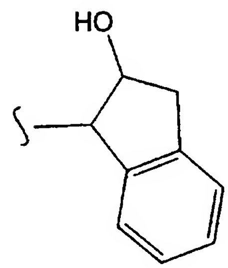
,

,

,



and

.

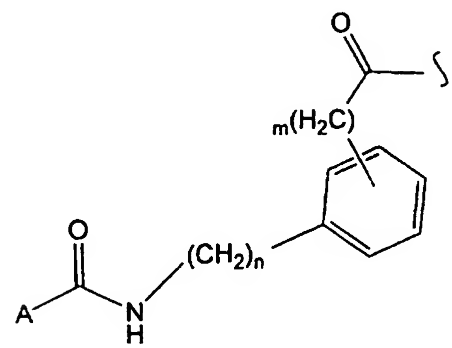
51. The method of Claim 49 wherein:

R^3 is 3-isoquinolinyl or 2-benzofuranyl; and

R^4 and R^5, taken together, form a hetercyclic ring selected from the group consisting of pyrollidine and substituted pyrrolidinyl, indoline, isomers of indoline, substituted indoline, substituted isomers of indoline, tetrahydroisoquinoline, substituted tetrahydroisoquinoline, tetrahydroquinoline, substituted tetrahydroquinoline, piperidone, substituted piperidone, piperidine, substituted

piperidines, tetrahydro-oxazines and substituted tetrahydro-oxazines.

52. The method of Claim 48 wherein R^1 is represented by the following structural formula:



wherein:

A is selected from the group consisting of an aryl group, a substituted aryl group, a heteroaryl group and a substituted heteroarylgroup; and

n and m are each zero or one.

53. A method of treating an individual suffering from a disease associated with leukocyte infiltration of tissues expressing the molecule MAdCAM-1, comprising administering a therapeutically effective amount of an inhibitor represented by the following structural formula:

$$R^1\text{-}X\text{-}Y'\text{-}Z\text{-}R^2$$

wherein:

Y' is a dipeptide $[AA]_1$-$[AA]_2$ having the sequence Asp-Thr;

X and Z are independently chosen from the group consisting of a covalent bond, an amino acid or a

peptide, wherein each amino acid in X and Z is independently selected from the group of naturally occurring amino acids;

R^1 is R^3-CO-;

R^2 is $-NR^4R^5$;

R^3 is selected from the group consisting of a lower alkyl, substituted lower alkyl, aryl, substituted aryl, heteroaryl and substituted heteroaryl; and

R^4 and R^5 are each independently selected from the group consisting of hydrogen, a lower alkyl group, a substituted lower alkyl group, an aryl group, a substituted aryl group, a heteroaryl group and a substituted heteroaryl group, wherein:

1) R^4 and R^5 are not both -H; and
2) taken together, R^4 and R^5 can form a heterocyclic ring; and

taken together, X, Y' and Z form a peptide containing no more than about fifteen amino acids; and

wherein the peptide formed from X, Y' and Z is optionally cyclized with the proviso that, if the peptide formed from X, Y' and Z is cyclized, the nitrogen at the N-terminus of Y' is not bonded to a glycine or a sarcosine.

54. The method of Claim 53 wherein R^3 is selected from the group consisting of triphenylmethyl, diphenylmethyl, 3,5-diphenylphenyl, 2-furanyl, 3-furanyl, 9-xanthenemethyl, 2,2,2-triphenylethyl, 2-anthracene, methyl, cyclopentyl, 2-indolyl, 2-indanyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl, 3-benzothienyl, cyclohexyl, 5-phenylpentyl, 4-isobutyl-α-methylphenylmethyl, 4-biphenylmethyl, α-naphthylmethyl, 4-heptylphenyl, phenylmethyl, *trans* 2-phenylethenyl and 2,2,3,3-tetramethylcyclopropyl.

55. The method of Claim 54 wherein R^4 and R^5 are each independently selected from the group consisting of -H, 2-hydroxyethyl, benzyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl, 3-benzothienyl, $-CH_2$-2-thienyl, $-CH_2$-3-thienyl, $-CH_2$-2-furanyl, $-CH_2$-3-furanyl, 3,4-dimethoxybenzyl, and isopentyl.

56. The method of Claim 53 wherein:

R^3 is selected from the group consisting of diphenylmethyl, triphenylmethyl, *trans* 2-phenyl-ethylenyl, 2-phenyl-ethynyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl and 3-benzothienyl;

R^4 is selected from the group consisting of 2-hydroxyethyl, benzyl, 2-benzofuranyl, 3-benzofuranyl, 2-benzothienyl, 3-benzothienyl, $-CH_2$-2-thienyl, $-CH_2$-3-thienyl, $-CH_2$-2-furanyl, $-CH_2$-3-furanyl;

and R^5 is -H.

57. The method of Claim 56 wherein X and Z are each a covalent bond.

58. The method of Claim 53 wherein the peptide formed from X, Y and Z is cyclized.

59. The method of Claim 53 wherein the disease is selected from the group consisting of inflammatory bowel disease and insulin-dependent diabetes mellitus.

60. A method of inhibiting the binding of a cell expressing a ligand for MAdCAM-1 on the cell surface to MAdCAM-1 or a portion thereof, comprising contacting the cell with an effective amount of an inhibitor represented by the following structural formula:

$$R^1\text{-}X\text{-}Y\text{-}Z\text{-}R^2$$

wherein:

Y is a pentapeptide $[AA]_1$-$[AA]_2$-$[AA]_3$-$[AA]_4$-$[AA]_5$ wherein:

$[AA]_1$ is selected from the group consisting of leucine, cysteine, aspartic acid, glutamic acid, isoleucine, alanine, valine, glycine, N-methylleucine, serine, threonine, ornithine and lysine;

$[AA]_2$ is selected from the group consisting of aspartic acid, glutamic acid, phenylalanine and tyrosine;

$[AA]_3$ is selected from the group consisting of threonine, serine, valine, proline and 4-hydroxyproline;

$[AA]_4$ is selected from the group consisting of serine, cysteine, aspartic acid, glutamic acid, proline, 4-hydroxyproline, threonine, valine, isoleucine, alanine, glycine, ornithine and lysine; and

$[AA]_5$ is selected from the group consisting of leucine, isoleucine, N-methylleucine, threonine, ornithine, serine, valine, alanine, glycine, phenylalanine, cysteine, aspartic acid, glutamic acid and lysine;

X and Z are independently chosen from the group consisting of a covalent bond, an amino acid or a peptide, wherein each amino acid in X and Z is independently selected from the group of naturally occurring amino acids;

R^1 is R^3-CO-;

R^2 is $-NR^4R^5$;

R^3 is selected from the group consisting of a lower alkyl, substituted lower alkyl, aryl, substituted aryl, heteroaryl and substituted heteroaryl; and

R^4 and R^5 are each independently selected from the group consisting of hydrogen, a lower alkyl group, a substituted lower alkyl group, an aryl group, a substituted aryl group, a heteroaryl group and a substituted heteroaryl group, wherein:

1) R^4 and R^5 are not both -H; and
2) taken together, R^4 and R^5 can form a heterocyclic ring; and

taken together, X, Y and Z form a peptide containing no more than about fifteen amino acids; and

wherein optionally the peptide formed from X, Y and Z is cyclized.

61. The method of Claim 60 wherein Y is a pentapeptide $[AA]_1$-$[AA]_2$-$[AA]_3$-$[AA]_4$-$[AA]_5$ having the sequence Leu-Asp-Thr-Ser-Leu (SEQ ID NO: 1) with the proviso that any single one of $[AA]_1$, $[AA]_2$, $[AA]_3$, $[AA]_4$ or $[AA]_5$ can vary, being any naturally occurring amino acid.

62. The method of Claim 61 wherein the ligand is human $\alpha 4\beta 7$ integrin.

63. The method of Claim 62 wherein the cell is a leukocyte.

64. The method of Claim 63 wherein MAdCAM-1 is expressed on the surface of an endothelial cell.

65. The method of Claim 60 wherein the peptide formed from X, Y and Z is cyclized.

66. A method of inhibiting the binding of a cell expressing a ligand of MAdCAM-1 to MAdCAM-1 or a portion thereof, comprising contacting the cells with an inhibitory amount of a compound represented by the following structural formula:

$$R^1\text{-}X\text{-}Y'\text{-}Z\text{-}R^2$$

wherein:

Y' is a dipeptide $[AA]_1$-$[AA]_2$ having the sequence Asp-Thr or a tripeptide $[AA]_1$-$[AA]_2$-$[AA]_3$ having the sequence Leu-Asp-Thr;

X and Z are independently chosen from the group consisting of a covalent bond, an amino acid or a peptide, wherein each amino acid in X and Z is independently selected from the group of naturally occurring amino acids;

R^1 is R^3-CO-;

R^2 is $-NR^4R^5$;

R^3 is selected from the group consisting of a lower alkyl, substituted lower alkyl, aryl, substituted aryl, heteroaryl and substituted heteroaryl; and

R^4 and R^5 are each independently selected from the group consisting of hydrogen, a lower alkyl group, a substituted lower alkyl group, an aryl group, a substituted aryl group, a heteroaryl group and a substituted heteroaryl group, wherein:

1) R^4 and R^5 are not both -H; and
2) taken together, R^4 and R^5 can form a heterocyclic ring; and

taken together, X, Y' and Z form a peptide containing no more than about fifteen amino acids; and

wherein optionally the peptide formed from X, Y' and Z is cyclized with the proviso that, if the peptide formed from X, Y' and Z is cyclized and if Y' is Asp-Thr, the nitrogen at the N-terminus of Y' is not bonded to a glycine or a sarcosine.

67. The method of Claim 66 wherein the ligand is human α4β7 integrin.

68. The method of Claim 67 wherein the cell is a leukocyte.

69. The method of Claim 68 wherein MAdCAM-1 is expressed on the surface of an endothelial cell.

70. The method of Claim 66 wherein the peptide formed from X, Y' and Z is cyclized.

71. A compound represented by the following structural formula:

$$R^1\text{-}Y'\text{-}R^2$$

wherein:

Y' is a dipeptide $[AA]_1$-$[AA]_2$ having the sequence Asp-Thr;

R^1 is R^3-CO-;

R^2 is $-NR^4R^5$;

R^3 is selected from the group consisting of a lower alkyl group, a substituted lower alkyl group, an aryl group, a substituted aryl group, a heteroaryl group and a substituted heteroaryl group; and

R^4 and R^5 are each independently selected from the group consisting of hydrogen, a lower alkyl group, a substituted lower alkyl group, an aryl group, a substituted aryl group, a heteroaryl group and a substituted heteroaryl group, wherein:

1) R^4 and R^5 are not both -H; and

2) taken together, R^4 and R^5 can form a heterocyclic ring.

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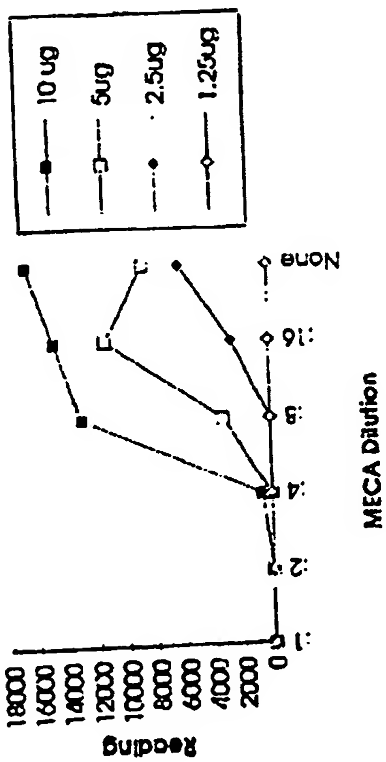
10 ug
5ug
2.5ug
1.25ug
Reading
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
MECA Dilution
None

Figure 1

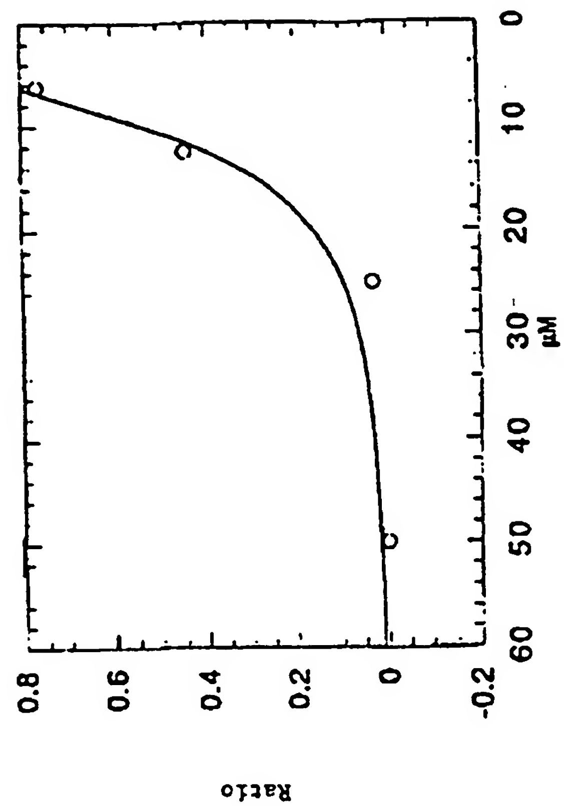

Figure 2

```
BamHI
GGA TTC ATG GAA TCC ATC CTG------------------------------------
         M   E   S   I   L

        Signal Peptide of MAdCAM-1 . . .


        Complete Extracellular Domain of Murine MAdCAM-1
-----------------------------//-------------------------------


                                    BamHI
---CCG AAT TCC TCC TCC ACC GGA TCC GCT GAT GCT GCA CCA ---------
    P   N   S   S   S   T   G   S   A   R   A   A   P . . .
                                    Murine Cκ . . .
```

Figure 3

```
ATGGATTTCGGACTGGCCCTCCTGCTGGCGGGGCTTCTGGGGCTCCTCCTCGGCCAGTCCCTCCAGGTGAAGCCCCTGCA 80
 M  D  F  G  L  A  L  L  L  A  G  L  L  G  L  L  L  G  Q  S  L  Q  V  K  P  L  Q
GGTGGAGCCCCCGGAGCCGGTGGTGGCCGTGGCCTTGGGCGCCTCGCGCCAGCTCACCTGCCGCCTGGCCTGCGCGGACC 160
  V  E  P  P  E  P  V  V  A  V  A  L  G  A  S  R  Q  L  T  C  R  L  A  C  A  D
GCGGGGCCTCGGTGCAGTGGCGGGGCCTGGACACCAGCCTGGGCGCCGTGCAGTCGGACACGGGCCGCAGCGTCCTCACC 240
R  G  A  S  V  Q  W  R  G  L  D  T  S  L  G  A  V  Q  S  D  T  G  R  S  V  L  T
GTGCGCAACGCCTCGCTGTCGGCGGCCGGGACCCGCGTGTGCGTGGGCTCCTGCGGGGGCCGCACCTTCCAGCACACCGT 320
 V  R  N  A  S  L  S  A  A  G  T  R  V  C  V  G  S  C  G  G  R  T  F  Q  H  T  V
GCAGCTCCTTGTGTACGCCTTCCCGGACCAGCTGACCGTCTCCCCAGCAGCCCTGGTGCCTGGTGACCCGGAGGTGGCCT 400
  Q  L  L  V  Y  A  F  P  D  Q  L  T  V  S  P  A  A  L  V  P  G  D  P  E  V  A
GTACGGCCCACAAAGTCACGCCCGTGGACCCCAACGCGCTCTCCTTCTCCCTGCTCGTCGGGGGCCAGGAACTGGAGGGG 480
C  T  A  H  K  V  T  P  V  D  P  N  A  L  S  F  S  L  L  V  G  G  Q  E  L  E  G
GCGCAAGCCCTGGGCCCGGAGGTGCAGGAGGAGGAGGAGGAGCCCCAGGGGGACGAGGACGTGCTGTTCAGGGTGACAGA 560
 A  Q  A  L  G  P  E  V  Q  E  E  E  E  E  P  Q  G  D  E  D  V  L  F  R  V  T  E
GCGCTGGCGGCTGCCGCCCCTGGGGACCCCTGTCCCGCCCGCCCTCTACTGCCAGGCCACGATGAGGCTGCCTGGCTTGG 640
  R  W  R  L  P  P  L  G  T  P  V  P  P  A  L  Y  C  Q  A  T  M  R  L  P  G  L
AGCTCAGCCACCGCCAGGCCATCCCCGTCCTGCACAGCCCGACCTCCCCGGAGCCTCCCGACACCACCTCCCCGGAGCCT 720
E  L  S  H  R  Q  A  I  P  V  L  H  S  P  T  S  P  E  P  P  D  T  T  S  P  E  P
CCCAACACCACCTCCCCGGAGTCTCCCGACACCACCTCCCCGGAGTCTCCCGACACCACCTCCCAGGAGCCTCCCGACAC 800
 P  N  T  T  S  P  E  S  P  D  T  T  S  P  E  S  P  D  T  T  S  Q  E  P  P  D  T
CACCTCCCAGGAGCCTCCCGACACCACCTCCCAGGAGCCTCCCGACACCACCTCCCCGGAGCCTCCCGACAAGACCTCCC 880
  T  S  Q  E  P  P  D  T  T  S  Q  E  P  P  D  T  T  S  P  E  P  P  D  K  T  S
CGGAGCCCGCCCCCCAGCAGGGCTCCACACACACCCCCAGGAGCCCAGGCTCCACCAGGACTCGCCGCCCCTGAGATCTCC 960
P  E  P  A  P  Q  Q  G  S  T  H  T  P  R  S  P  G  S  T  R  T  R  R  P  E  I  S
CAGGCTGGCCCCACGCAGGGAGAAGTGATCCCAACAGGCTCGTCCAAACCTGCGGGTGACCAGCTGCCCGCGGCTCTGTG 1040
 Q  A  G  P  T  Q  G  E  V  I  P  T  G  S  S  K  P  A  G  D  Q  L  P  A  A  L  W
GACCAGCAGTGCGGTGCTGGGACTGCTGCTCCTGGCCTTGCCCACGTATCACCTCTGGAAACGCTGCCGGCACCTGGCTG 1120
  T  S  S  A  V  L  G  L  L  L  L  A  L  P  T  Y  H  L  W  K  R  C  R  H  L  A
AGGACGACACCCACCCACCAGCTTCTCTGAGGCTTCTGCCCCAGGTGTCGGCCTGGGCTGGGTTAAGGGGGACCGGCCAG 1200
E  D  D  T  H  P  P  A  S  L  R  L  L  P  Q  V  S  A  W  A  G  L  R  G  T  G  Q
GTCGGGATCAGCCCCTCCTGAGTGGCCAGCCTTTCCCCCTGTGAAACCAAAATAGCTTGGACCCCTTCAAGTTGAGAACT 1280
 V  G  I  S  P  S
GGTCAGGGCAAACCTGCCTCCCATTCTACTCAAAGTCATCCCTCTGCTCACAGAGATGGATGCATGTTCTGATTGCCTCT 1360

TTGGAGAAGCTCATCAGAAACTCAAAAGAAGGCCACTGTTTGTCTCACCTACCCATGACCTGAAGCCCCTCCCTGAGTGG 1440

TCCCCACCTTTCTGGACGGAACCACGTACTTTTTACATACATTGATTCATGTCTCACGTCTCCCTAAAAATGCGTAAGAC 1520

CAAGCTGTGCCCTGACCACCCTGGGCCCCTGTCGTCAGGACCTCCTGAGGCTTTGGCAAATAAACCTCCTAAAATGATAA 1600

AAAAAAAAAAAAAAAAAAAAAAAA 1624
```

Figure 4

ATGGATTTCGGACTGGCCCTCCTGCTGGCGGGGCTTCTGGGGCTCCTCCTCGGCCAGTCCCTCCAGGTGAAGCCCCTGCA 80
M D F G L A L L L A G L L G L L L G Q S L Q V K P L Q

GGTGGAGCCCCCGGAGCCGGTGGTGGCCGTGGCCTTGGGCGCCTCGCGCCAGCTCACCTGCCGCCTGGCCTGCGCGGACC 160
V E P P E P V V A V A L G A S R Q L T C R L A C A D

GCGGGGCCTCGGTGCAGTGGCGGGGCCTGGACACCAGCCTGGGCGCGGTGCAGTCGGACACGGGCCGCAGCGTCCTCACC 240
R G A S V Q W R G L D T S L G A V Q S D T G R S V L T

GTGCGCAACGCCTCGCTGTCGGCGGCCGGGACCCGCGTGTGCGTGGGCTCCTGCGGGGGCCGCACCTTCCAGCACACCGT 320
V R N A S L S A A G T R V C V G S C G G R T F Q H T V

GCAGCTCCTTGTGTACGCCTTCCCGGACCAGCTGACCGTCTCCCCAGCAGCCCTGGTGCCTGGTGACCCGGAGGTGGCCT 400
Q L L V Y A F P D Q L T V S P A A L V P G D P E V A

GTACGGCCCACAAAGTCACGCCCGTGGACCCCAACGCGCTCTCCTTCTCCCTGCTCGTCGGGGGCCAGGAACTGGAGGGG 480
C T A H K V T P V D P N A L S F S L L V G G Q E L E G

GCGCAAGCCCTGGGCCCGGAGGTGCAGGAGGAGGAGGAGGAGCCCCAGGGGGACGAGGACGTGCTGTTCAGGGTGACAGA 560
A Q A L G P E V Q E E E E E P Q G D E D V L F R V T E

GCGCTGGCGGCTGCCGCCCCTGGGGACCCCTGTCCCGCCCGCCCTCTACTGCCAGGCCACGATGAGGCTGCCTGGCTTGG 640
R W R L P P L G T P V P P A L Y C Q A T M R L P G L

AGCTCAGCCACCGCCAGGCCATCCCCGTCCTGCACAGCCCGACCTCCCCGGAGCCTCCCGACACCACCTCCCCGGAGTCT 720
E L S H R Q A I P V L H S P T S P E P P D T T S P E S

CCCGACACCACCTCCCCGGAGTCTCCCGACACCACCTCCCAGGAGCCTCCCGACACCACCTCCCCGGAGCCTCCCGACAA 800
P D T T S P E S P D T T S Q E P P D T T S P E P P D K

GACCTCCCCGGAGCCCGCCCCCCAGCAGGGCTCCACACACACCCCCAGGAGCCCAGGCTCCACCAGGACTCGCCGCCCTG 880
T S P E P A P Q Q G S T H T P R S P G S T R T R R P

AGATCTCCCAGGCTGGGCCCACGCAGGGAGAAGTGATCCCAACAGGCTCGTCCAAACCTGCGGGTGACCAGCTGCCCGCG 960
E I S Q A G P T Q G E V I P T G S S K P A G D Q L P A

GCTCTGTGGACCAGCAGTGCGGTGCTGGGACTGCTGCTCCTGGCCTTGCCCACCTATCACCTCTGGAAACGCTGCCGGCA 1040
A L W T S S A V L G L L L L A L P T Y H L W K R C R H

CCTGGCTGAGGACGACACCCACCCACCAGCTTCTCTGAGGCTTCTGCCCCAGGTGTCGGCCTGGGCTGGGTTAAGGGGGA 1120
L A E D D T H P P A S L R L L P Q V S A W A G L R G

CCGGGCAGGTCGGGATCAGCCCCTCCTGAGTGGCCAGCCTTTCCCCCTGTGAAAGCAAAATAGCTTGGACCCCTTCAAGT 1200
T G Q V G I S P S

TGAGAACTGGTCAGGGCAAACCTGCCTCCCATTCTACTCAAAGTCATCCCTCTGTTCACAGAGATGGATGCATGTTCTGA 1280

TTGCCTCTTTGGAGAAGCTCATCAGAAACTCAAAAGAAGGCCACTGTTTGTCTCACCTACCCATGACCTGAAGCCCCTCC 1360

CTGAGTGGTCCCCACCTTTCTGGACGGAACCACGTACTTTTTACATACATTGATTCATGTCTCACGTCTCCCTAAAAATG 1440

CGTAAGACCAAGCTGTGCCCTGACCACCCTGGGCCCCTGTCGTCAGGACCTCCTGAGGCTTTGGCAAATAAACCTCCTAA 1520

AATGAAAAAAAAAAAAAAA 1539

Figure 5